IMMUNE SYSTEM STATUS, SELECT NUTRIENT INTAKES, AND MICRONUTRIENT STATUS IN YOUNG WOMEN WITH A CHRONIC SUBOPTIMAL ENERGY INTAKE

by

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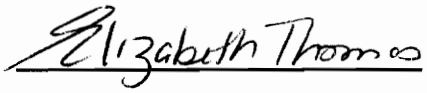

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(ABSTRACT)

Little is known regarding the nutrition and health implications of chronic dieting in college-women. Therefore, this study was conducted to determine nutrient intakes, zinc status, and immune system status in 19-24 year-old college women consuming different energy intakes. A suboptimal group (SG) (n=9), with a chronic suboptimal energy intake of $\leq$70% of the RDA, was matched for partial energy output to a control group (CG) (n=9), with a chronic optimal energy intake of $\geq$90% of the RDA. Zinc status was assessed using plasma zinc, red blood cell (RBC) zinc, and RBC fragility. Immune system status was assessed using IgG, IgM, C3, % T cell, and % lymphocyte. The SG consumed significantly lower intakes of macronutrients and several micronutrients than the CG ($p<.05$). Nutrient intakes in the SG, $\leq$70% of the RDA, were energy, carbohydrate, fat, vitamin D, calcium, iron, zinc, and copper; but only vitamin D and zinc in the CG. Zinc status and immune system status were not significantly different between the two groups ($p>.05$). No correlations were found between zinc intake and the zinc status markers, suggesting that the markers were not

sensitive indicators. In the SG only, significant positive correlations were found between intakes of energy, macronutrients, and zinc, and one or more of the immune components ($p<.05$). These findings suggest that although the apparent immune system status was not altered by a suboptimal energy intake, in an inadequate energy intake, immune system status reflected nutrient intakes.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
B cell	Bursa-Derived Cell
CG	Control Group
C3	Complement 3
DTH	Delayed-Type Hypersensitivity
EEA	Energy Expenditure for Activity
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IRB	Institutional Review Board
MANOVA	Multivariate Analysis of Variance
PBS	Phosphate Buffered Saline
PCM	Protein-Calorie Malnutrition
PEM	Protein-Energy Malnutrition
RBC	Red Blood Cell
RDA	Recommended Dietary Allowances
REE	Resting Energy Expenditure
SG	Suboptimal Group
T cell	Thymus-Derived Cell

CHAPTER I

INTRODUCTION

Adequate nutrition is essential for all physiological functions in the body. Therefore, a lack of adequate nutrition would alter, and likely impair, body functions. However, it is unclear what extent of a nutrient reduction would be necessary for an impairment in function to occur. Aside from poverty stricken regions, most individuals in the US do not have severe nutrient deficiencies, although some select populations are found to chronically consume inadequate or "suboptimal" nutrition when compared to the Recommended Dietary Allowances (Hernon et al. 1986, Alaimo et al. 1994, McDowell et al. 1994). The nutrients most often found to be inadequate in these populations are energy (total calories) and select micronutrients. The significance of mild to moderate nutrient deficiencies resulting from suboptimal nutritional intake is uncertain, partially because there is often a lack of clinical symptoms associated with moderate nutritional deficiencies. However, a lack of clinical symptoms does not warrant a lack of concern. On the contrary, the consequences of chronically consuming inadequate nutrients may be of great concern. One possible concern is the effect of suboptimal nutrition on health status.

Post-adolescent females are one select group that may be at particular risk for the potential health problems resulting from nutritional deficiencies. Currently, our society dictates the ideal body image as having a very thin, angular body shape; and young females, in particular, experience the greatest social pressure to obtain and maintain that image. However, the method

most often used by young women to reach and maintain that image is chronic calorie restriction or "chronic dieting", increasing the potential for nutritional deficiencies (Connor-Greene 1988, Moore 1988, Bellisle et al. 1995). Various researchers have shown that the nutrient intakes of post-adolescent females are chronically inadequate to meet their nutritional needs, particularly for the at risk nutrients (Murphy et al. 1986, Horwath 1991, Bailey 1989).

Zinc, a multifunctional trace element, is one micronutrient that is consistently deficient in the diet of post-adolescent females (Horwath 1991, Block et al. 1993). Yet, as with most other micronutrients, the physiological consequences of a suboptimal zinc intake and/or status are uncertain. Part of the uncertainty lies in the identification of zinc status, which is difficult due to a lack of sensitive and specific biochemical indicators of zinc.

Health status is often determined by measuring one or more components of the immune system. Several biochemical and functional tests can be used to assess health (Roitt 1994). Nutritional status influences the health status of an individual in that adequate nutrients are essential for the proper functioning of the immune system; and, because of the strong correlation between nutritional status and health status, immune components and function have often been assessed in determining nutritional status (Linn 1987, Marcos et al. 1993).

Research has focused exclusively on the immunological consequences of severe malnutrition, including only those populations considered at high risk for immune system impairment (Forse et al. 1981, Thompson et al. 1987, Chandra 1983). To what extent a chronic suboptimal nutritional intake or

nutritional status would affect immune system functions is unclear. Also unknown, but essential, is the effect of suboptimal nutrition on future health status. Such as with nutritional assessment, immune status assessment is difficult. Individual immune indicators often do not accurately represent immune status because the immune system is a highly sensitive system, affected by the psychological as well as the physiological state of an individual (Friedlander et al. 1986). The purpose of this study was to determine what influence a chronic suboptimal energy intake has on individual nutrient intakes, zinc status, and immune system status in post-adolescent, college-age females.

Research Objectives

The specific objectives of the study were...

(1) to compare average macronutrient and select micronutrient intakes of a population of college-age females at a chronic suboptimal energy intake with college-age females at a chronic optimal energy intake.

(2) to compare zinc status in college-age females at a chronic suboptimal energy intake with college-age females at a chronic optimal energy intake.

(3) to compare indicators of the immune system in college-age females at a chronic suboptimal energy intake with college-age females at a chronic optimal energy intake.

Research Hypotheses

The following null hypotheses were tested.

Ho 1: There is no difference in the average macronutrient and select micronutrient intakes of college-age females at a chronic suboptimal energy intake as compared to college-age females at a chronic optimal energy intake.

Ho 2: There is no difference in the average zinc intake or zinc status of college-age females at a chronic suboptimal energy intake as compared to college-age females at a chronic optimal energy intake.

Ho 3: There is no difference in the serum concentrations of immune components of college-age females at a chronic suboptimal energy intake as compared to college-age females at a chronic optimal energy intake.

CHAPTER II

LITERATURE REVIEW

Prevalence of Dieting in Women

Ideal body shape for women is defined by society. In this society, the ideal shape has evolved into a thin, almost angular type physique (Garner et al. 1980). Yet, while the ideal body shape has decreased in size over the past few decades, the average woman has increased in size (Garner et al. 1980, Wiseman et al. 1992). In a study to investigate the change in the ideal feminine physique from 1959 to 1978, Garner et al. (1980), measured the changes in weight and body measurements of Playboy Playmates and Miss America Contestants. In addition to demonstrating an overall decrease in the size of the ideal physique, the investigators showed that the "ideal" females were 15% or more below their expected weight, a weight difficult to maintain through a normal diet. A 10 year follow-up to this study by Wiseman et al. (1992) reported similar findings of a continued decrease in the size of the "ideal" female, yet an increase in the size of the average female.

Because of society's pressures to be thin, women's desire to achieve the ideal physique is strong. Not being able to easily achieve the ideal physique has led to many women disliking their bodies, even if they are at a normal, healthy weight and body shape. Connor-Greene (1988) studied 100 college women's perceptions of their bodies and found that 88% of the college women desired to lose weight, although only 2% were actually overweight. In addition, 25% of the women who felt that they were at a normal weight,

still desired to lose weight. Such aspirations reflect the unrealistic views of women wanting to obtain the thin physique.

The methods used by women to achieve the ideal physique are concerning, mainly since the ideal is a size below what is considered healthy. Most women have difficulty achieving the thin size without employing extreme weight loss methods. Media has readily supplied much of the weight loss methods used by women. Over the last four decades the number of weight loss articles in popular magazines has significantly increased (Garner et al. 1980, Wiseman et al. 1992). However, often these weight loss techniques involve extreme caloric restriction. Nevertheless, women are using these techniques to obtain that ideal image, and maintaining the ideal image often requires maintaining the weight loss strategies, thus "chronic dieting" results.

Dieting in the young female population is prevalent. In a telephone survey by Serdula et al. (1994) of 7,173 women 18 to 29 years of age, 40% reported trying to lose weight. The number one technique used by 83% of those women trying to lose weight was caloric restriction.

The desire to achieve the ideal physique begins in adolescence. Young girls are instilled with the concepts of attractiveness, the most prominent concept being a slender body (Freedman 1984). The result of such beliefs initiate many women to begin dieting early in their lives. Moore (1988) investigated the prevalence of dieting in adolescent females and found that 63% of 12-15 year olds were dissatisfied with their bodies and 31% had used caloric restriction to lose or maintain weight. Similar results were found by Hill et al. (1992) in a study of 9 and 14 year old girls. In their investigation,

Hill et al. measured dietary restraint in relation to body esteem and body satisfaction. Restraint eating and body dissatisfaction were found equally in both age groups, indicating a desire to achieve the ideal physique regardless of age. Since the desire to achieve the ideal physique begins at young ages, it would be assumed that the weight loss practices, in particular chronic dieting, would also begin at young ages. Story et al. (1991) surveyed 17,471 females in grades 7 through 12 to determine the prevalence of dieting and chronic dieting in this young population. They found that 62% of the population had reported dieting in the previous year and another 12% of the population had reported dieting more than ten times in the previous year, which are those Story and colleagues classified as chronic dieters. The investigators also found that the percentage of chronic dieters increased proportionally with age, indicating an increased preoccupation of dieting with age.

The practice of dieting is carried with women into adulthood. Society's pressure for thinness is experienced the most by college age women. As a result, the incidence of dieting in this age group is great (Salmons 1987, Grunewald 1985, Connor-Greene 1988, Peters et al. 1996). Salmons (1987) investigated the prevalence of dieting in 754 college women and found that 30% were actively controlling their weight, 94% of those by caloric restriction. Yet, only 2.5% were considered overweight. Grunewald (1985) reported similar findings. In 166 college females, 45.2% had reported dieting in the previous 8 months, another 18% reported chronically dieting. The 18% chronic dieters is greater than the percentages found by Story et al. (1991) in adolescents females, demonstrating the increase in the prevalence of chronic dieting in females as they enter adulthood.

Nutrient Intakes of Women

Since the prevalence of chronic dieting is high in young women, it is of concern that these women would be at risk for developing nutrient deficiencies. Several studies have established the theory that as total caloric intake decreases, individual nutrient intakes decrease proportionally (Bueckle et al. 1993, Hernon et al. 1986, Whitehead 1995). Therefore, chronic dieting or chronic caloric restriction puts the young woman at risk for developing multiple nutritional deficiencies resulting from an inadequate caloric intake.

Macronutrient and micronutrient intakes have been studied in various populations (Block et al. 1993, Nowak et al. 1988, Paterson et al. 1985). Yet, because of the high prevalence of dieting in young college females, the nutrient intakes and status of this particular population have been studied extensively (Bueckle et al. 1993, Bailey 1989, Hernon et al. 1986). Dieting prevalence and it's subsequent effects on nutrient intakes were reported by Bueckle et al. (1993) in a study of 158 college males and females. Bueckle and colleagues found a relationship between prevalence of dieting and nutrient intake in the college females, in that, as the incidence of dieting increased (chronic dieting), average energy intake decreased. Overall intakes of riboflavin, niacin, calcium, iron, copper, and zinc, were below the RDA for this female population. When grouped by caloric intake, the researchers found that females consuming less than 1200 kilocalories per day had significantly lower intakes of several vitamins and minerals than women consuming between 1200 and 1500 kilocalories per day. Yet, both groups were inadequate in total energy intake based on the recommended energy intakes

(NRC, 1989). Therefore, macronutrient and micronutrient intakes would have likely been found to be inadequate in both groups. This assumption is supported by the research of Bailey (1989) in an investigation of the dietary intakes of 59 college women. Mean caloric intake was 1550 kilocalories per day, or only 70% of the current RDA. Associated with the low energy intakes were low intakes of carbohydrate (76% of the RDA) and fat (73% of the RDA). Protein intake, however, was found to be more than adequate (143% of the RDA), yet protein intake is often found to be adequate even at suboptimal energy intakes. Regardless of protein intake, a protein deficiency would likely result from an inadequate energy intake, because the available protein would be used to supply energy to maintain body functions.

Micronutrient intakes are often inadequate to meet the nutritional needs of most college women, especially in women consuming inadequate energy intake. Hernon et al. (1986) grouped a population of college women by calorie intake; one group consuming less than 1200 kilocalories per day, the other group consuming more than 1200 kilocalories per day. Although both populations were found to be deficient in several micronutrients, those women consuming less than 1200 kilocalories per day had a greater number of nutrient deficiencies and/or a greater degree of nutrient deficiency as compared to the group consuming more than 1200 kilocalories per day. Average energy intake was only 48% of the RDA in the group consuming less than 1200 kilocalories per day, as compared to an average energy intake of 88% of the RDA in the group consuming greater than 1200 kilocalories per day. In addition to the deficit in energy, intakes of carbohydrate, fat, calcium,

iron, thiamin, riboflavin, and niacin fell below the recommended intakes in the group consuming less than 1200 kilocalories per day.

As demonstrated, deficiencies of macronutrient and micronutrient intake in college women is prevalent. To complicate the situation, some women combine physical activity with caloric restriction to increase weight loss (Serdula et al. 1994, Salmons 1987). Physical activity increases nutrient requirements; therefore, women combining caloric restriction with physical activity would likely increase their risk for developing nutrient deficiencies. Nowak et al. (1988) analyzed the dietary intakes of 10 female athletes and found that intakes of energy, carbohydrate, fat, vitamin D, vitamin C, folate, vitamin B6, calcium, iron, and zinc were below the recommended allowances. Of those, intakes of vitamin D, folate, vitamin B6, iron, and zinc were below 70% of the current RDA. Current recommendations consider only moderately active women, thus, the nutritional requirements of this sample would be greater. Therefore, the implications for women practicing caloric restriction and exercising frequently would be of an even greater concern than those practicing caloric restriction alone.

Not all researchers have found nutrient intakes in college women to be inadequate. Gottschalk et al. (1977) assessed the diets of 51 college females and found all nutrient intakes to be adequate, with the exception of iron, which was at 67% of the current RDA. Murphy et al. (1986) found similar results in 996, 18-24 year old, females surveyed in the second national health and nutrition examination survey (NHANES II) report. Only zinc, copper, and calcium were found to be inadequate (below 70% of the RDA). In both studies, inadequate nutrient intakes were defined as consuming less than 70%

of the recommended intake. In both studies, other nutrient intakes were reported that did not completely meet the RDA yet were not below 70% of the RDA. Therefore, the problem of inadequate nutrient intakes exists, but the extent of the problem lies in it's definition.

Nevertheless, several nutrients have been repeatedly found to be inadequate (<70% of the RDA) in college women's diets. The nutrients most often found to be inadequate are the B vitamins, particularly B6 and B12 (Horwath 1991, Block et al. 1993, Nowak et al. 1988), and the minerals calcium, copper, iron, and zinc (Hernon et al. 1986, Murphy et al. 1986, Block et al. 1993, Nowak et al. 1988).

Nutrient Status of Women

Nutrients interact in the body. Rarely is there a physiological process that requires a single nutrient to function. Therefore, measuring the status of a single nutrient is difficult, primarily because most status indicators lack sensitivity and/or specificity for a single nutrient. Hence, true status of a particular nutrient is often unknown. Most researchers rely on measuring nutrient concentrations in blood components to assess nutritional status. Measuring nutrient levels in blood components is a relatively quick, easy, and inexpensive assessment tool for determining status. Therefore, it is often the method of choice when determining nutrient status. Yet, the accuracy of using blood components to measure nutrient status may be limited. Horwath (1991) measured iron, vitamin A, and vitamin C intakes and corresponding levels of serum ferritin, plasma ascorbic acid, and plasma carotene as

measures of nutrient status in 84 college women. Intakes of vitamin A and vitamin C were 141% and 172% of the RDA, respectively. Corresponding mean blood values were 2.68 μmol/L for plasma carotene and 90 μmol/L for plasma ascorbic acid, both in the upper end of the normal value ranges. Iron intake was below recommendations (75% RDA) with a corresponding serum ferritin level of 31.5 μg/L, a value corresponding to the lower end of the normal value range. This study showed that blood levels adequately reflected nutrient status; and therefore, may be good indicators of status. However, other research has challenged these findings.

Jacques et al. (1993) investigated the correlation between 12 micronutrient intakes and their corresponding biochemical indicators of nutrient status in 139 adults, age 40-83 years of age. The results of the study varied. The nutrients folate, vitamin C, vitamin D, vitamin B12, vitamin E, and vitamin A were strongly correlated with their corresponding biochemical indicator; however, thiamin, riboflavin, vitamin B6, magnesium, and zinc were not correlated with their corresponding biochemical indicator. Therefore, even though blood levels may be adequate to represent status of some nutrients, they may not be adequate to represent all nutrients.

Zinc

Zinc, an essential trace element, affects many physiologic and metabolic processes in the body. Zinc functions as a component of more than 100 metalloenzymes and is a regulator of numerous biologic processes, some of which include protein metabolism, hormone and neurotransmitter function,

antioxidant function, and immune function (Walsh et al. 1994). In addition, research has pointed to an interesting role of zinc as an essential cofactor of many enzymes that regulate cell membrane structure and function (Betteger et al. 1981). The specific mechanisms by which zinc functions in these processes are largely unknown. Nevertheless, the importance of zinc to the body is certain, a certainty that has been established based on the multitude of reported deficiency effects associated with zinc (Wada et al. 1983).

The current recommended intake for zinc, for women age 19 to 24, is 12 mg per day (NRC 1989). Yet, as discussed previously, zinc is one of the minerals often found to be deficient in the diets of college women (Hernon et al. 1986, Murphy et al. 1986, Block et al. 1993, Nowak et al. 1988). The NHANES III report of zinc intake for 838 women age 20 to 29 demonstrates this deficiency. Based on 24-hour dietary recalls, mean zinc intake was determined to be 9.7 mg per day, or 80% of the current RDA, which is above the 70% suboptimal range but below the recommended intake (Alaimo et al. 1994). An inadequate zinc intake puts college women at risk for developing an inadequate zinc status and the resulting deficiency associated symptoms.

Zinc Deficiency Symptoms

The clinical manifestations resulting from a zinc deficiency have been extensively studied (Wada et al. 1983, Prasad 1985a). The degree of the manifestation depends primarily upon the length and severity of the deficiency. Some overt symptoms that have been noted in a chronic and/or a severe zinc deficiency include growth retardation, anorexia, delayed sexual

maturity, immune impairment, delayed wound healing, and thyroid atrophy (Wada et al. 1983, Prasad 1985a, Aggett et al. 1995).

Many of the specific biochemical and functional changes occurring in a zinc deficiency have been identified through zinc depletion/repletion studies. A depletion/repletion study conducted by Baer and colleagues (1985) helped define symptoms of a severe acute zinc deficiency. Six healthy young men, age 21-30, were fed severely zinc deficient diets (0.28mg/day) for a period of 4 to 9 weeks, following which 3 of the 6 men were repleted (6.0, 23.2, or 46.3 mg/day) for 2 to 5 weeks. Measures were made on several clinical, biochemical, and functional parameters thought to be zinc associated. By the end of the depletion period, subjects had experienced some of the associated symptoms of zinc deficiency including diarrhea, skin problems, significantly decreased white blood cell and lymphocyte counts, significantly decreased activity of numerous zinc-dependent enzymes including alkaline phosphatase and lactic dehydrogenase, and significantly decreased taste acuity and glucose tolerance. In the repletion period, only the enzyme activity measures returned to baseline.

The effects of a severe zinc deficiency are concerning; yet, only a small percentage of the population experience severe zinc deficiencies. On the contrary, more common is chronic, mild to moderate zinc deficiencies (Walsh et al. 1994). The symptoms associated with a milder zinc deficiency are less known. However, it would be reasonable to assume that some of the effects seen in a severe zinc deficiency also would be seen in a mild zinc deficiency.

Prasad et al. (1978) described the effects of a mild chronic zinc deficiency followed by zinc repletion in 4 adult males. Two subjects were fed diets containing 2.7 mg of zinc per day for 24 weeks followed by a 12-week period of 30 mg of zinc per day. The remaining two subjects were fed diets containing 3.5 mg of zinc per day for a period of 50 weeks, followed by an 8-week period of 30 mg of zinc per day. All subjects declined in measures of plasma, RBC, leukocyte, and urinary zinc by the end of the depletion period. In addition, a decreased activity of several zinc-dependent metabolic enzymes including alkaline phosphatase, thymidine kinase, and lactic dehydrogenase occurred. Upon repletion, all measures returned to normal or above normal levels, suggesting that zinc was the specific cause of the decreased measures.

How then do these deficiency associated symptoms relate to the normal population? Several select groups are at risk for developing a zinc deficiency. Those groups considered at risk include infants and children, vegetarians, and the elderly (Swanson et al. 1979). Also, included are those individuals found to chronically consume inadequate energy intake or "chronic dieters". Little research has focused on the implications of a zinc deficiency in chronic dieters. However, zinc
deficiency-related manifestations in the elderly have been observed. Prasad and colleagues (1993) conducted a study of 180 free-living elderly adults and found the mean zinc intakes to be inadequate (69% of the RDA). In addition, assessment of several zinc-related measures were made and compared to normal values reported for young adults. Significantly lower levels of zinc in blood fluids and cells were found, as well as decreased immune function, as measured by interleukin activity, thymulin activity, and response to

antigens. These findings could be explained as age-related decline in function. However, zinc supplementation in 13 of the elderly subjects refuted that explanation through increased blood levels of zinc and improvement of the functional measures of immunity following zinc supplementation. Therefore, mild zinc deficiency has potentially severe implications, particularly for chronic dieters who are often found to consume inadequate zinc.

Assessment of Zinc Status

Zinc is primarily located in pools throughout the body. Minute amounts of zinc also are contained in extracellular blood fluids. Since zinc is primarily located in inaccessible pools throughout the body, assessing zinc status is difficult. Therefore, zinc is often measured in plasma or other blood components that can be easily assessed. Yet, in a zinc deficiency, blood components are often not affected until the deficiency has been well established (Aggett et al. 1995). This phenomenon occurs because body pools release zinc into extracellular blood compartments to maintain blood levels, in the process depleting the body pools (Aggett et al. 1995, Prasad 1985a). Therefore, analyzing zinc blood levels may not adequately reflect an acute zinc deficiency. However plasma zinc may be applicable in identifying a chronic zinc deficiency. Lowered plasma zinc levels have been seen in several studies involving long-term zinc depletion (Baer et al. 1984, Ruz et al. 1992, Prasad et al. 1978).

Measuring zinc content in blood cells also has been proposed as a possible zinc status indicator. RBCs and leukocytes have been suggested as potential zinc status indicators (Prasad et al. 1978, Wallwork 1987, Prasad 1985b, Ruz et al. 1992). In a study previously reviewed, Prasad et al. (1978) determined that RBC zinc content was depressed following zinc depletion; however, because of the long half-life of the RBC, the depression was not evident until the zinc deficiency was well established. Therefore, such as with plasma zinc, RBC zinc content may not adequately identify an acute zinc deficiency but could identify a chronic zinc deficiency. Leukocytes, however, may adequately identify an acute zinc deficiency. In the same study, leukocytes, which have a shorter half-life, responded quickly to the zinc deficiency, which was seen in the decreasing leukocyte levels early into the zinc depletion. Neutrophils and lymphocytes also have been assayed as potential zinc status indicators (Prasad 1985b, Prasad et al. 1993, Baer et al. 1985). However, more research is needed regarding utilizing these blood cells as potential indicators of zinc status.

Hair, urine, semen, saliva, and fecal zinc also have been proposed as potential zinc status indicators (Ruz et al. 1992, Verus et al. 1994, King 1986, Baer et al. 1984). Baer and colleagues (1984) assessed these biochemical markers as potential zinc status indicators in their depletion/repletion study, previously reviewed, involving 6 young men fed severe zinc deficient diets (0.28mg/day) followed by zinc repletion diets (6.0, 23.2, or 46.3 mg/day). Hair and salivary zinc content did not change in the study, however, urinary zinc, fecal zinc, and semen zinc declined. Yet, only urinary zinc responded quickly to the zinc repletion, suggesting that urinary zinc may be a sensitive indicator

of zinc status. However, a study by Verus et al. (1994) disputed urinary zinc as a good indicator of status. In the study, urinary zinc excretion was measured in 24 males following consumption of a 50 or 100 mg zinc supplement. Changes in urinary zinc were seen only in the higher supplement intake, suggesting that extreme changes in dietary zinc are needed to produce significant changes in urinary zinc levels.

A few zinc-dependent enzymes have been proposed as possible functional indicators of zinc status. (Prasad et al. 1978, Prasad 1985b, Ruz et al. 1992, Baer et al. 1985, Tamura et al. 1996). Several studies show that decreases in the activities of alkaline phosphatase and lactate dehydrogenase have resulted from a depletion of zinc (Prasad et al. 1978, Baer et al. 1985). Furthermore, following zinc repletion, the activities of these enzymes rapidly returned to normal, demonstrating that they would be sensitive zinc status indicators. However, a study by Bales et al. (1994) found no changes in alkaline phosphatase activity in their moderate zinc depletion/repletion study. Measuring the activity of Angiotensin-Converting Enzyme (ACE), a zinc-dependent enzyme, also has been proposed as a potential functional indicator of zinc status. Yet, a recent study by Tamura et al. (1996) found no changes in ACE activity in a zinc-deficient population supplemented with zinc for a 20-week period, suggesting that ACE would not be a sensitive functional indicator of zinc status.

As stated previously, zinc is essential in the maintenance of RBC membrane integrity. Thus, measuring RBC fragility as a potential indicator of zinc status is reasonable. Many studies have demonstrated that zinc deficiency induces cell membranes to become fragile (O'Dell et al. 1987,

Record et al. 1990). O'Dell and colleagues (1987) demonstrated this effect of zinc deficiency on RBC fragility in rats, where a severe zinc deficiency caused RBCs to lyse easier in saline solutions. Furthermore, following repletion with zinc, RBC fragility decreased. Yet, many other nutrients are involved in the maintenance of RBC membranes. Therefore, assessing RBC fragility as a measure of zinc status would not be specific to zinc alone.

Overview of the Immune System

The immune system is a complex network of cells that function as the body's defense mechanism against foreign invaders known as antigens. White blood cells, also know as leukocytes, are the defense cells in the immune system. Polymorphonuclear and mononuclear cells are the major classes of white blood cells. Mononuclear cells constitute the major, specific defense cells in immunity and are generally comprised of four main types of cells: bursa-derived cells (B cells), thymus-derived cells (T cells), macrophages, and natural killer cells (NK cells) (Elgert 1996). All immune cells have specific functions, which interact to defend the body against foreign invaders.

The immune system's response to an antigen is comprised of two distinct but interacting immune system components: innate and acquired immunity. Innate immunity, termed such because it is the immune defenses that you are born with, is a basic, nonspecific line of defense, working mainly to prevent antigens from entering the system. Acquired immunity is the second type of immunity, developing throughout the life of an individual following exposure to antigens. Unlike innate immunity, acquired

immunity is more specific in response to an antigen and is able to "remember" antigen exposures to provide better defenses with future exposures.

Acquired immunity is divided into two sublevels: humoral and cell-mediated immunity. Humoral immunity functions mainly in destroying extracellular antigens. The major defense cells of humoral immunity are the B cells which, upon antigen invasion, selectively transform into a new cell called a plasma cell. The plasma cell produces proteins called antibodies, who's major classification is the immunoglobulins (Ig). There are five classes of Igs: IgG, IgM, IgD, IgE, and IgA; each with distinct functions in immune response. The primary function of Igs is in activating "killer cells" (mast cells, macrophages, and NK cells) and activating other immune components that also promote killing activity. The second sublevel of acquired immunity is cell-mediated immunity. T cells are the major defense cells of cell-mediated immunity. There are several subclasses of T cells that serve distinct functions, ultimately regulating T cell responses. Additionally, T and B cells have specificity to antigens and upon stimulation by an antigen, proliferate rapidly for a strong defense. Macrophages and NK cells are the two other major types of immune cells that function primarily in the direct killing of antigens.

Another system involved in immune response is the complement system, a series of proteins that function by "activation reaction", ultimately activating certain complement proteins that promote antigen killing. Complement (C) proteins are identified by numbers, with C3 as the most abundant complement protein in the system.

The immune system is an interwoven defense system where the proper function of each immune cell group relies on the proper functioning of other immune cell groups. Each specific group of immune cells contributes to the overall defense response. Thus, proper functioning of all immune cell groups is crucial for the entire defense system to work. Therefore, an impairment of any single cell group has the potential to lower the entire immune response, putting the individual at risk for an illness.

Assessment of Immune System Status

Assessing status of the immune system is difficult without considering how the immune system functions. Previously discussed was a generalized overview of the major cells and proteins of the immune system and how they function in an immune response. From that discussion, it should be apparent that assessing the status of the immune system would be difficult, primarily because of the complexity of immune components and of the immune response. Nevertheless, techniques have been developed to roughly estimate the health status of individuals. Determining immune system status is generally accomplished through the assessment of blood levels of immune components or by assessing immune responsiveness to antigenic substances.

A complete blood count with differential (CBCD) is commonly used to determine the concentration of select immune cells in the blood. A CBCD identifies blood levels of general categories of leukocytes, including total lymphocytes. Immune status is then determined by comparing the CBCD

values to normal blood levels. A CBCD does not identify specific populations of immune cells such as T and B cells and their subsets. The determination of these cells is achieved by using specialized techniques (Roitt 1994). Antibody and complement levels also are assessed, using specialized techniques, as determinants of immune status.

Functional measures of immunity have been employed as indicators of immune status. Delayed-type hypersensitivity (DTH) is the most commonly used functional test for assessing immune status (Linn 1987, Chandra et al. 1980, Forse et al. 1981). DTH is a relatively simple test that measures immune response to antigen exposure.

These assays and similar ones are the primary tools used in assessing immunity. However, interpreting the results of these assays must be accomplished with consideration to the complexity and sensitivity of the immune system. The immune system is responsive to numerous factors other than illness. Environmental and psychological stress can alter immunocompetence (Friedlander 1986). Nutritional status also has been shown to influence immunity (Friedlander 1986, Chandra 1983, Thompson et al. 1987). Therefore, these conditions must be considered when interpreting the results of immune assays. Nevertheless, assessing immune cell and protein levels in blood and immune responsiveness to antigens are essentially ideal for determining health status.

Nutrition and Immunity

Macronutrients and Immunity

As previously mentioned, nutritional status is one factor that influences immunity. The influence of nutrition on the immune system has been extensively studied; and, immune system status has even been proposed as a potential indicator of nutritional status (Chandra et al. 1980, Chandra 1991). Adequate nutrition is essential in the proper functioning of the immune system. The importance of adequate nutrition for immune function has been demonstrated by the severity of immune-related deficiencies occurring in malnourished individuals, most notably in those individuals with Protein-Energy Malnutrition (PEM). PEM, also referred to as Protein-Calorie Malnutrition (PCM), is predominantly seen in underdeveloped nations. PEM is defined as a severe lack of total calories and, consequently, a severe lack of the essential macronutrients and micronutrients.

PEM's influence on immunocompetence has been widely studied (Good et al. 1992; Sakamoto et al. 1992; Forse 1994; Chandra 1981,1983, 1989, 1991; Garre et al. 1987, Fakhir et al. 1989). PEM is associated with the suppression of many immune functions including depressed cell-mediated immunity, particularly a reduction of the numbers of circulating T cells and T cell subsets (Fakhir et al. 1989, Good et al. 1992, Forse 1994, Garre et al. 1987), and a reduction of T cell responsiveness to antigenic substances (Forse 1994,

Garre et al. 1987, Chandra 1981). In PEM, a reduction in antibody responsiveness to antigens also has been observed (Moulias et al. 1985, Garre et al. 1987). However, circulating antibody levels are generally increased in malnutrition due to repeated infections occuring with PEM (Forse 1994, Chandra 1989). Complement levels in circulation also have been found to be reduced in PEM (Sakamoto et al. 1992, Forse 1994, Sirisinha et al. 1973, Chandra 1975).

The effect of single macronutrient deficiencies on the immune system has not been widely studied; primarily because, in the malnourished state, rarely is there a deficiency of a single macronutrient. As stated before, a decrease in total caloric intake is accompanied by a proportional decrease in individual macronutrient intakes. However, the potential roles of protein, carbohydrate, and fat in immunity have been reviewed. Adequate levels of protein are necessary for synthesis of the immune components. A study by Castaneda and collegues (1995) measured concentrations of T cells and immunoglobulins along with DTH response in 12 elderly women consuming either a marginal protein intake (0.45 g protein/kg body weight) or an optimal protein intake (0.92 g protein/kg body weight). In the marginally deficient group, T cell and immunoglobulin concentrations did not change by the end of the depletion period, while DTH response decreased from baseline levels. However, in the optimal group, T cell and immunoglobulin levels increased and DTH response also improved. Therefore, these results had two findings: (1) that protein intake in this elderly population was marginal at the start of the study; and, (2) that protein is essential to the maintenance of immune cells and their functions.

Adequate intakes of carbohydrate and fat also are necessary to maintain proper function in the immune system. Carbohydrate's direct effect on immunity has not been assessed. However, carbohydrates would essentially provide the energy needed for proper immune function and immune cell synthesis. Fat has multiple roles in immunity. Lipids are essential for the integrity of immune cell membranes, and for the formation of lipoproteins that regulate specific immune cells functions (Johnston 1988, Chandra 1983).

Micronutrients and Immunity

The essentiality of micronutrients to immune functions has been primarily assessed through analyzing the effects of malnutrition on immunity. As with macronutrients, single micronutrient deficiencies are rare; and therefore, little information from malnutrition studies pertains to the effects of individual micronutrient deficiencies on immunity. Furthermore, the specific mechanisms by which micronutrients function in the immune system are unknown. However, theories of how select micronutrients affect immunity have been postulated based on the known functions of micronutrients and based on studies involving micronutrient depletion/repletion effects on the immune system. These studies have resulted in an assessment of the generalized effects of micronutrient deficiencies on the immune system (Keusch 1990, Chandra 1990, Sherman 1992, Bendich 1993, Beisel 1982). Deficiencies of micronutrients affect all levels of immunity, from immune cell development through immune response to antigens (Beisel 1982). Yet, certain micronutrients have multiple

effects on immunity; and therefore, are considered more critical to immune system function. Those nutrients, which exhibit multiple effects on immune function, include antioxidants, iron, copper, and zinc (Bendich 1993, Lukasewycz et al. 1990, Spenser et al. 1990, Daudu et al. 1994).

Zinc and Immunity

Zinc is one of the micronutrients that has been found to be multifunctional in immunity. As previously discussed, zinc is an essential trace element that functions in numerous enzymatic reactions and is essential to the integrity of cell membranes. The effects of a zinc deficiency have been discussed. The immunological impacts resulting from zinc deficiencies demonstrate the roles of zinc in immune functions. Zinc is involved in the maintenance and function of many immune components including lymphocyte, antibody and natural killer cells (Fraker et al. 1986). The exact mechanisms by which zinc functions in immunity are unclear, but it has been postulated that zinc's role is in the synthesis, maturation and maintenance of immune components; and, as an essential cofactor to many enzymes regulating immune responses to antigens (Fraker et al. 1986, Keen et al. 1990, Miller et al. 1992). It is because of these multiple functions that zinc is critical to the immune system.

The roles of zinc in immunity have been demonstrated in various studies through assessing the immunological changes resulting from a zinc deficiency. In a study, previously reviewed, Baer and colleagues (1985) assessed immunocompetence in 6 men following a severe acute zinc

depletion. White blood cells and lymphocyte numbers were reduced, suggesting the role of zinc in the synthesis, maturation, and maintenance of immune cells. Zinc's role in immunity also was demonstrated in the previously reviewed study conducted by Prasad et al. (1993). In the study, Prasad and colleagues determined that zinc deficiency caused a suppression of immune function and response; in particular, a decreased interleukin activity (an immune response to an antigen exposure) and thymulin activity (a thymus hormone regulating T cell maturation).

Caloric Restriction and Immunity

Previously discussed was the influence of severe malnutrition, or PEM, on the immune system. Yet, as stated previously, PEM is predominately found in developing nations. Possibly more prevalent in the US is mild to moderate macronutrient and micronutrient deficiencies associated with caloric restriction. Also previously discussed, was the prevalence of caloric restriction or "dieting" in college women desiring to lose weight. The effects of caloric restriction on the immune system are not as well known as the effects of PEM. However, that does not mean that caloric restriction and the potentially associated nutritional deficiencies do not influence immunocompetence. On the contrary, in recent years, several studies have looked at the how acute moderate caloric restriction affects immune functions (Kelley et al. 1994, Nieman et al. 1996, McMurray et al. 1990, Field et al. 1991).

A study by Nieman et al. (1996) demonstrated the effects of moderate caloric restriction on immune function. Thirteen obese women were put on a 1200-1300 kilocalorie per day diet for 12 weeks. Lymphocyte proliferation response to antigens; circulating numbers of total leukocytes, monocytes, T cells, B cells, and natural killer cells; phagocytosis; and active oxidative leukocyte burst (a response to antigens) were measured. At the end of 12 weeks, the obese women had lost an average of 9.9 kilograms and significant declines were seen in total leukocytes, monocytes, and natural killer cells, but not in T and B cells. In addition, lymphocyte proliferation response and active oxidative burst declined. The researchers concluded that the weight loss, not the obesity or caloric restriction, had caused the decline in immune function.

A study by Kelley et al. (1994) also looked at the immunological effects of caloric restriction in 10 overweight women. The women were placed on micronutrient supplemented 1300 kilocalorie per day diets for 84 days and monitored for changes in IgG, IgA, IgM, C3, C4, lymphocytes and their subsets, circulating NK cells, DHT response, and peripheral blood mononuclear cell proliferation response to antigens. Subjects lost an average of 8 kilograms during the study period. Significant decreases in serum concentrations of IgG, IgA, C3 and circulating NK cells were observed by the end of the study period. The researchers also made note of the health status of the subjects throughout the study period; however, they found no incidence of illness in the women. The researchers concluded that a restricted energy intake was the cause of the impaired immunity, not weight loss as determined by Nieman et al. (1996).

The two studies demonstrate the negative effects of a moderate energy restriction on immunity. Micronutrient supplementation in the study by Kelley et al. (1994) excluded the possibility of a micronutrient deficiency influencing immune status. Therefore, most likely, the immunological impact was a result of a restriction of energy or other macronutrient intake; or, as postulated by Nieman and colleagues, the result of weight loss. In both studies, overweight or obese women were assessed and caloric restriction was short term. The majority of the college women dieting to achieve the ideal physique are at normal weight. In addition, the caloric restriction or dieting used by these women is often chronic. Therefore, in college women, there is the potential for developing a severely compromised immune system as a result of dieting, and that potential could be heightened with chronic dieting.

CHAPTER III

METHODS

Subjects

Female subjects were recruited from the Virginia Polytechnic Institute and State University Campus and from the surrounding Blacksburg area. Flyers (Appendix A) posted throughout the campus and community advertised for participation in a study of nutrition, health, and exercise. Flyers also were presented in several nutrition and exercise classes and student athletic and social organizations. The flyer contained the initial criteria of being between the ages of 19 and 24 and having maintained current body weight (within a five pound margin) for at least the past 12 months.

Women interested in the study were asked to contact the primary investigator by phone. During the phone interview, additional information regarding the goals and the purpose of the study was given, the initial criteria of age and body weight was verified, and the potential subjects were informed of their participation responsibilities in the study. Women expressing further interest were scheduled for an initial interview.

Potential subjects, meeting inclusion criteria, were accepted into the study based on their average energy intake. Participants were identified in the study as either having at a chronic suboptimal energy intake (suboptimal group), with an average daily energy intake of less than or equal to 24.5 kilocalories per kilogram of body weight (representing 70% or less of the Recommended Energy Intake for females between 19 and 24 years of age); or,

identified as having at a chronic optimal energy intake (control group) with an average daily energy intake of greater than or equal to 34.0 kilocalories per kilogram of body weight (representing 90% or more of the Recommended Energy Intake for females between 19 and 24 years of age) (NRC 1989). Suboptimal and control subjects were matched for partial energy output, which included resting energy expenditure (REE) and energy expenditure for activity (EEA).

Experimental Protocol

Subjects were continuously recruited from March through October of 1996. The protocol for screening and participation in the study was identical for all participants. The time period for subject involvement in the study from the initial telephone contact through the final blood collection varied for each subject, however, the average time for each subject to complete the study was two months. A flowchart schematic of the study design is shown in Figure 1.

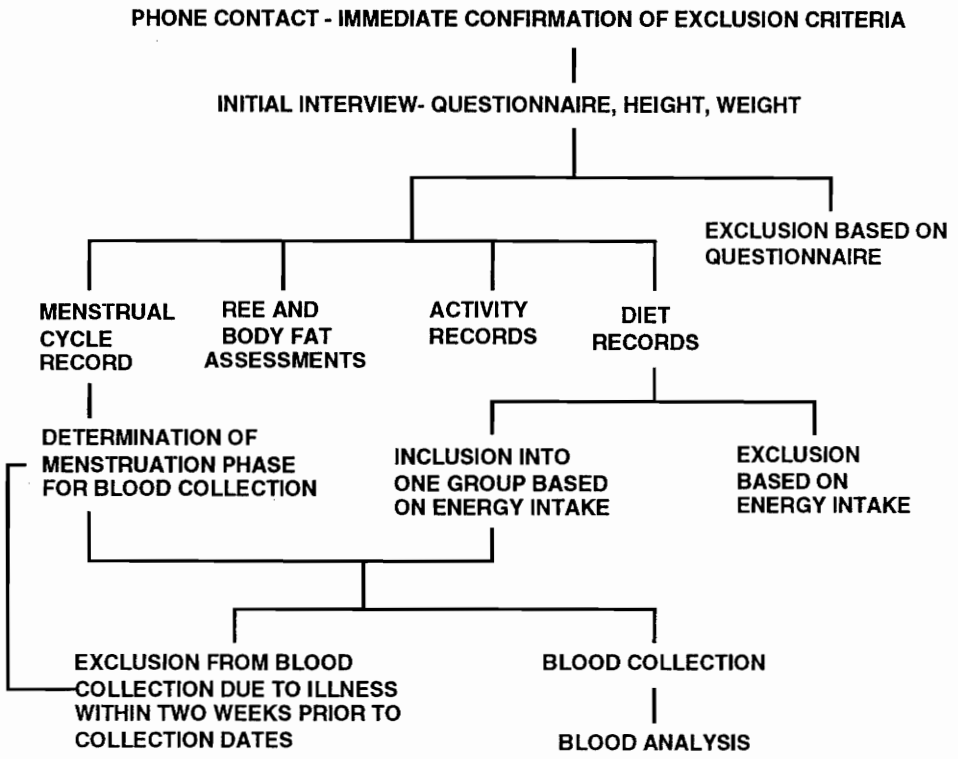

Figure 1. Flowchart of study design.

Initial Interview

The initial interview consisted of a questionnaire (Appendix B) comprised of subject descriptive and exclusion criteria questions which was adapted from a questionnaire used by Lewis (1996). Exclusion from participation in the study occurred with any of the following conditions: past diagnosed eating disorder; indication of a current, diagnosable eating disorder; history of a chronic disease or illness; irregularity of the menstrual cycle; and/or chronic use (defined as multi-weekly) of any illicit, prescription, or "over-the-counter" drugs (except oral contraceptives and alcohol) that had the

potential of affecting any of the parameters measured. A current, diagnosable eating disorder was identified using specific questions obtained from the questionnaire designed by Lewis (1996). The questions were developed from the diagnostic criteria of eating disorders outlined in the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (1994). Other questions were included to collect descriptive information, an exercise history, a 24-hour diet record, and information regarding eating habits of the potential subjects. Upon completion of the questionnaire, subjects expressing concerns about the interview questions related to the determination of eating disorders were supplied with an eating disorder information sheet developed by Lewis (1996), which included phone numbers where they could obtain more information (Appendix C).

Informed consent was obtained from each potential participant following the completion of the interview questionnaire. All potential subjects read and signed the Informed Consent form and use of human subjects for this study was approved by the Institutional Review Board (IRB) of Virginia Polytechnic Institute and State University (Appendix D).

Dietary Assessment

Upon meeting the criteria for participation in the study, potential subjects were asked to complete a 14-day diet record. The 14 days of diet recording was the minimum number of days, determined by Basiotis et al. (1987), that would represent the true average caloric intake of an individual. Subjects were supplied with verbal and written instructions (Appendix E) to

aid in recording their diet. Schematic diagrams of food portions and sizes (Appendix F) along with an example of the food record (Appendix G) were given to the potential subjects to improve the accuracy of the diet records. The diet record consisted of three sections: recording the food (or beverage) and it's description and/or preparation method (if needed), recording the quantity consumed, and recording the size (if applicable). A space for comments was included on each record for any additional information the potential subject wanted to supply.

The Nutritionist III^{TM}, Version 7.2, software for Macintosh (N-Squared Computing, Salem, OR) was used for the analysis of the diet records. All entries were made by the same investigator to minimize error. Substitutions of food items not included in the software package were made using similar food items. An average daily intake of energy (total calories), macronutrients, and select micronutrients was determined for each potential subject using the 14-day records.

Energy Expenditure for Activity Assessment

Energy expenditure for activity (EEA), measured in kilocalories, was determined for each subject using the exercise history information supplied in the initial questionnaire and by using a 14-day record of intentional activity completed by each of the subjects (Appendix H). Verbal and written instructions (Appendix E) were given to each subject to aid in recording intentional activity. The type, intensity, and duration of each activity

included in the records was used to estimate the subject's EEA by using the following equation obtained from the research of Ainsworth et al. (1993).

Kilocalories = [mets* x body wt (kg)] x [minutes of exercise/60]

*mets = the measure of the intensity of an activity equal to the ratio of work metabolic rate to resting metabolic rate

A final estimation of the kilocalories expended per week was determined for each subject by summing the two week's individual activity assessments. The two weeks were averaged to obtain a final weekly EEA value. A daily EEA value was determined for each subject and combined with the value of REE for subject matching between the two groups.

Resting Energy Expenditure Assessment

Resting Energy Expenditure (REE), a measure of the minimum amount of energy required to sustain body functions, was determined for each subject following a 12-hour food and exercise fast. A laboratory assistant, LeaAnn Fritsch, performed all REE assessments, which were determined using indirect calorimetry, a measure of oxygen consumption using open circuit spirometry on a Medical Graphics CPX/D metabolic cart (Medical Graphics, Minneapolis). For the assessment, subjects rested in a dark room in a supine position for 25-30 minutes, following which, subjects were fitted with a mouthpiece and pneumotach. Expired gases were collected at 8 second

intervals for a 20 minute period, with the initial 10 minutes used to equilibrate the equipment and the final 10 minutes to determine the REE.

A predicted REE was determined using the Harris Benedict Prediction Equation which was a program in the Medical Graphics CPX/D metabolic cart software (Medical Graphics, Minneapolis). The program was used to predict REE values for each subject based on sex, height, weight, age, and body surface area. The actual REE value and the predicted REE value were used to calculate a value of the percent of the predicted REE. The actual REE value was combined with the determined EEA value for a final measure of daily partial energy output for each subject.

Anthropometric Data

Subjects' height and weight were measured during the initial interview and during the REE assessment. Percent body fat was calculated from each subject using skin-fold thickness measures. Duplicate skin-fold measures were taken at three anatomical locations on the body, including the triceps, thigh, and suprailliac regions. Measures were summed and percent body fat was determined using the following equation from Jackson et al. (1985).

$$\text{Body Density} = 1.0994921 - 0.0009929(X_1) + 0.0000023(X_1)^2 - 0.0001392(X_2)$$

Where X_1 = sum of the triceps, thigh, and suprailium skin-folds

X_2 = age in years

Blood Collection and Processing

Whole blood samples were collected twice during the first eight days following the onset of the subject's menstrual cycle to avoid hormone fluctuations influencing the biochemical assays. To ensure this, menstrual chart records (Appendix I) were kept by each subject and used to predict the appropriate time for blood collections.

Subjects were monitored for health status for the two-week period prior to blood collections in an attempt to prevent any potential influence an illness may have on the parameters measured. Two weeks prior to the blood collections, subjects were instructed to contact the primary investigator if they became ill at any time before the collection dates. A final check of health status was made on each day preceding a blood collection date. If the subject experienced any symptoms of a prolonged illness (illness for more than one day) in the two weeks prior to collection, blood collections were postponed and rescheduled for the following menstrual cycle, and the process was repeated.

Twice, during the first 8 days of the subject's menstrual cycle, approximately 25 mls of whole blood were collected following a 10 to 12 hour fast. Janet Rinehart, MLT, ASCP, performed all of the phlebotomy procedures in the study. The blood was collected by venipuncture into a 9.5 ml SST Gel and Clot Activator tube, a 5 ml 15% EDTA (K_3) solution tube, and a 7 ml Trace Element-Free Sodium Heparin tube. Each subject was allowed to rest for 10 minutes following collection and provided with juice and snacks.

Samples were allowed to set for a period of 10 to 45 minutes following blood collections. An aliquot of the 5 mls of whole blood collected in the 15% EDTA (K_3) solution tube was transferred to a separate tube for use in the determination of Percent Thymus Cell (% T cell). The remaining 4 mls of whole blood was sent to the Carilion Consolidated Laboratory in the Roanoke Memorial Hospital (Roanoke, Virginia) for a Complete Blood Count with Differential (CBCD) analysis.

An aliquot of the blood collected in the Trace Element-Free Sodium Heparin tube was transferred to a separate tube for use in the determination of Red Blood Cell (RBC) fragility. The remaining whole blood was centrifuged at 2500 rpm for 15 minutes at 4° C to separate the plasma. Aliquots of the plasma were stored in 8 ml test tubes at -20° C. The buffy coat was removed and the red blood cells were washed using 0.15 M NaCl and recentrifuged at 2500 rpm for 15 minutes at 4° C. The red blood cells were then lysed by the addition of deionized water (1: 1.4) and the lysate was stored at -80° C. All materials used in the preparation and storage of the plasma and red blood cells were acid washed with 2N HCL to prevent zinc contamination.

Serum was separated from the whole blood, collected in the SST Gel and Clot Activator tube, by centrifugation at 2800 rpm for 15 minutes at 5° C. One ml of the serum was sent to the Carilion Consolidated Laboratory for determination of Immunoglobulin G (IgG), Immunoglobulin M (IgM), and Complement 3 (C3) concentrations. The remaining serum was aliquoted in one ml increments into micro vials and stored at -20° C.

Serum Total Protein Determination

Serum total protein concentration was determined using a modification of a quantitative, colorimetric procedure (No. 541) purchased from Sigma Diagnostics (St. Louis, MO). The measure of serum total protein was based on a biuret reaction, with a color change resulting from the reaction of serum proteins and copper ions. The resulting color intensity was proportional to the total protein concentration.

Protein standards in concentrations of 2, 4, 6, and 8 mg/dl were made from the protein standard (Sigma Diagnostic, No. 540-10) diluted with phosphate buffered saline (PBS). Standards, control (Accutrol™ Chemistry Control, Sigma Diagnostics, No. A-2034), and serum samples were diluted 1:5 with PBS. In a 96 well microplate, 200 µls of Total Protein Reagent (Sigma Diagnostics, No. 541-2) was mixed separately with 20µls of the PBS diluted standards, control, or serum samples. PBS also served as the reagent blank. Solutions were incubated at room temperature for 10 minutes to allow for a complete color reaction to occur. Absorbance was read at 540 nm and concentrations were calculated using the Ceres 900 HDi Scanning Autoreader (Bio-tek Instruments, Winooski, Vt.), using the reagent blank as the reference.

Plasma and Red Blood Cell Zinc Determination

Zinc concentrations of plasma and red blood cell lysate were determined using Flame Atomic Absorption Spectroscopy on a Perkin-Elmer

Model 503 Atomic Absorption Spectrophotometer (AAS) following the procedure of Smith et al. (1979). Plasma and red blood cell lysate were diluted 1:3 and 1:10, respectively, with deionized water and absorbance was read at 213.8 nm using an air/acetylene flame. A working curve was established from zinc standards prepared in 5% glycerol/water (v/v) solutions at 100, 200, 500, and 1000 µg/L. A 5% glycerol/water (v/v) solution was used as a blank. Plasma and red blood cell lysate zinc concentrations were calculated directly from the curve. Pooled plasma was assayed at the beginning and end of each of the plasma and red blood cell zinc determinations as a measure of inter-assay reproducibility.

Red Blood Cell Fragility Determination

Red Blood Cell Fragility was measured using a modification of the procedures of Baker et al. (1962) and Cartwright (1963). Red blood cells are maintained in normal saline concentrations of approximately 0.85% NaCl at a pH of 7.4. A reduction in the NaCl concentration causes cells to lyse. The degree of lysis at varying NaCl concentrations indicates the fragility of the cells. Hemoglobin released from the lysed cells is measured spectrophotometrically at 540 nm. In the procedure, 50 µls of stored whole blood (at 2°C for approximately 4 hours) were added to 5 mls of sodium chloride concentrations of 0.00%, 0.10%, 0.20%, 0.30%, 0.35%, 0.40%, 0.45%, 0.50%, 0.55%, 0.65%, 0.75%, 0.85%. The solutions were allowed to incubate at room temperature for 30 minutes, following which, they were centrifuged at 1500 rpm for 5 minutes at 25° C. Two hundred microliters of each

supernatant were transferred to a microplate and absorbance was immediately read at 540 nm using the Ceres 900 HDi Scanning Autoreader (Bio-tek Instruments, Winooski, Vt.). The 0.85% NaCl concentration sample was used as the blank, where no lysis occurred. Percentage red blood cell lysis was calculated for each NaCl concentration using the following equation. The 0.00% NaCl solution represented complete lysis.

$$\% \text{ Lysis} = \frac{\text{Abs NaCl solution}}{\text{Abs } 0.00\% \text{ NaCl solution}} \times 100\%$$

Percent Thymus Cell (%T cell) Determination

Percent T cell was determined using Flow Cytometry. The procedure and materials used to prepare the samples were purchased from Becton Dickinson Immunocytometry Systems (San Jose, Ca.). The procedure for preparation of T cells was based on the binding ability of fluorescently-labeled monoclonal antibodies to specific cell surface antigens located on T cells. Whole blood was treated with a red blood cell lysing solution and centrifuged at 2100 rpm for 5 minutes at 2-8° C. The white blood cell pellet and residual debris was washed with PBS and recentrifuged. The pellet was resuspended in a 1% PBS with a protein carrier (2% w/v) and stained with fluorescein isothiocyanate (FITC)-labeled, CD3 (Leu-4) monoclonal antibody (Cat No. 92-0001). The solutions were allowed to incubate in a dark ice bath (0° C) for 30

minutes to allow binding to occur. FITC-labeled, Mouse IgG_1 was used as the monoclonal control for each sample analyzed (Cat No. 349041).

Samples and controls were analyzed immediately or preserved with 2% paraformaldehyde for later analysis (up to 5 days). All analyses were made at the Virginia-Maryland Regional College of Veterinary Medicine Flow Cytometry Laboratory using a Coulter Epics XL Flow Cytometer (Coulter Company, Opa Loc, Fl.) with XL software. Joan Kalnitsky, a flow cytometry technician, performed all of the analyses. Using the computer program, an area encompassing the T cell population was isolated from the remaining white blood cells and debris. Within a specific area, 2500 cells were counted, including the fluoresced T cells. A %T cell was calculated based on a proportion of the number of fluoresced T cells counted to the total number of cells counted.

Statistical Analysis

Descriptive statistics were used to compare the two groups in select behavioral, nutritional, and health related questions obtained from the initial interview questionnaire.

A student's t-test was used to compare the two groups for age, height, weight, % body fat, % of predicted REE, energy, macronutrient intakes, and micronutrient intakes (Microsoft Excel Version 4.0, Microsoft Corporation, Redmond, WA).

Multivariate analysis of variance (MANOVA) was used to compare biochemical indicators of zinc status and immune status using the statistical

package Minitab (Minitab Release 8, Minitab Inc., State College, Pa.). Measures included in the statistical analysis of zinc were plasma zinc concentration, RBC zinc concentration, and % lysis of RBCs at 0.30%, 0.35%, 0.40%, 0.45%, and 0.50% NaCl concentration. Measures included in the statistical analysis of immune status were % lymphocyte, % T cell, IgG, IgM, and C3 concentrations.

Correlation and regression analysis was used to determine correlations and p values between energy, macronutrient, and zinc intakes and the biochemical markers of the immune system and of zinc status. An alpha level of 0.05 was used to measure significance in all analyses.

CHAPTER IV

RESULTS

In the eight month recruitment period, 70 women were interviewed. Of those 70 women, 49 met all of the inclusion criteria and completed diet, activity and menstrual chart records. Twenty-eight of those women met the criteria for acceptance into one of the two groups based on their energy intake. Of those 28 women, 18 completed all of the components of the study.

Nine subjects were identified as having a chronic suboptimal energy intake (SG) and matched, within an 11% margin of total EEA and REE, with 9 subjects identified as having a chronic optimal energy intake (CG). The matching of subjects by EEA and REE is detailed in Table 1. Anthropometric measures of the two groups are shown in Table 2.

Mean age, height, and % of predicted REE were not significantly different between the two groups ($p>0.05$). Body weight of the SG appeared to be greater than the CG, although the difference was not significant. However, % body fat was significantly higher in the SG ($p<0.001$). Weight and % body fat was significantly positively correlated in the SG ($r= 0.916$, $p= 0.0003$) but not significantly correlated in the CG ($r= 0.536$, $p=0.13$). In association with the weight and body composition findings, 67% of the SG reported being at least "somewhat overweight" as compared to only 22% of the CG. Furthermore, 78% of the SG was at least "moderately dissatisfied" with the way their body was proportioned, as compared to only 22% of the CG. Possibly, as a result of dissatisfaction, 78% of the SG reported having been on a diet in their life, 29%

of those reported having been on 11 to 20 diets since they began dieting. In contrast, only 44% of the CG reported to have ever dieted.

Table 3 lists mean energy, macronutrient, and select micronutrient intakes of the two groups. Also included is the recommended dietary intakes for young women age 19-24. The SG consumed significantly lower amounts of total energy than the CG ($p<.001$). Intakes of protein, carbohydrate, fat, vitamin A, vitamin D, vitamin C, riboflavin, calcium, iron, and zinc were significantly lower in the SG group than in the CG ($p<0.05$). The SG failed to meet the recommended intakes for most of the nutrients observed. Nutrient intakes deficient in the SG were total energy, carbohydrate, fat, vitamin D, vitamin B6, calcium, iron, zinc, and copper. In comparison, the CG did not meet the recommended intakes for vitamin D, calcium, and zinc. Nutrient intakes less than 70% of the RDA are considered suboptimal. The SG consumed less than 70% of the RDA for energy, carbohydrate, fat, vitamin D, calcium, iron, zinc, and copper, where as only vitamin D and zinc intakes were suboptimal in the CG.

Eating habits of the two groups were assessed in the initial interview questionnaire. Seventy eight percent of the SG reported that they normally eat breakfast every day as compared to only 44% of the CG. In addition, 78% of the SG reported normally eating after 8 pm at least 3 nights per week, as compared to 56% of the CG. Yet, the majority of both groups (56%) reported that dinner was their largest meal of the day.

Table 4 shows mean values for plasma and RBC zinc concentrations, and % lysis of RBCs at varying NaCl concentrations. Also included in Table 4, are normal adult values for plasma zinc and RBC zinc (Wada et al. 1983) and

% lysis of RBCs (Baker et al. 1962). No significant difference in zinc status was found between the two groups.

Results of the biochemical measures of immune status are reported in Table 5. Also shown in Table 5, are normal adult values for IgG, IgM, C3, % T cells and % lymphocytes. No significant difference was found between SG and CG when all immune measures were compared as a group. In addition to the immune measures, frequency of illness was assessed from responses obtained from the interview questionnaire. Seventy eight percent of the CG and 89% of the SG reported to have been ill "less than monthly" in the previous year.

Tables 6 through 11 show the correlations between energy, macronutrient, and zinc intakes with the individual biochemical markers of the immune system and zinc status.

In the SG, significant positive correlations were found between total kilocalorie intake and IgM concentration ($p<.0008$) and %T cell ($p<.003$). However, in the CG, only a nonsignificant positive correlation was found between caloric intake and IgM concentration ($p<.08$). As a combined group, a significant positive correlation occurred between caloric intake and IgM concentration ($p<.01$).

Protein intake was significantly positively correlated with % Lymphocyte ($p<.05$), and somewhat positively correlated with IgG concentration ($p<.07$) in the SG. However, no significant correlations were found between protein intake and any of the immune markers in the CG, nor when the two groups were combined.

Carbohydrate intake was significantly positively correlated with IgM concentration ($p<.005$), and with % T cell ($p<.005$) in the SG. As with protein, no significant correlations were found between carbohydrate intake and the immune markers in the CG. Yet, one significant positive correlation, between carbohydrate intake and IgM concentration, was found when the two groups were combined ($p<.02$).

In the SG, fat intake was significantly positively correlated with IgM concentration ($p<.02$), and with % T cell ($P<.007$) and somewhat positively correlated with C3 concentration ($p<.08$). However, fat intake was not correlated with any immune measures in the CG. As a combined group, a significant positive correlation was found between fat intake and IgM concentration ($p<.03$).

Although neither correlations were significant, zinc intake was positively correlated with % T cell ($p<.06$) and somewhat positively correlated with % Lymphocyte ($p<.10$) in the SG. No significant correlations were seen between zinc intake and the immune measures in the CG. When the groups were combined, significant positive correlations were observed between zinc intake and IgM concentration ($p<.04$) and % T cell ($p<.03$).

No correlations were seen in either individual group or as a combined group when zinc intake was compared to plasma zinc concentrations, RBC zinc concentrations, or % lysis at the varying NaCl concentrations.

Table 1. Partial energy output and matching of subjects.

Suboptimal Group				Control Group				
Subject	REE (kcal/d)	EEA (kcal/d)	Total (kcal/d)	Subject	REE (kcal/d)	EEA (kcal/d)	Total (kcal/d)	margin of matching
#311	1001	49	1050	#109	1025	90	1115	6 %
#504	1140	103	1243	#107	1106	66	1172	6 %
#309	1268	142	1410	#120	1269	101	1370	3 %
#131	1279	133	1412	#112	1132	356	1488	5 %
#122	1260	219	1479	#104	1327	237	1564	6 %
#119	1222	326	1548	#100	1389	191	1580	2 %
#407	1371	328	1699	#305	1454	181	1635	4 %
#503	1465	288	1753	#304	1462	280	1742	1 %
#505	1563	407	1970	#302	1456	322	1778	11 %

Table 2. Anthropometric characteristics of subjects (mean ± S.D.)

Variable	Suboptimal Group (SG) (n=9)	Control Group (CG) (n=9)
Age (yrs)	20.7 ± 1.3	21.3 ± 2.0
Height (in)	65.7 ± 2.1	65.5 ± 3.0
Weight (kg)	63.6 ± 8.6	56.4 ± 8.8
% Body Fat	23.3 ± 3.1[a]	18.1 ± 3.1[b]
% Pred REE	87.6 ± 10.5	91.8 ± 8.9
kcal/kg bd wt	19.3 ± 4.1[a]	37.7 ± 3.6[b]

[a,b] $p<.001$

Table 3. Energy, macronutrient, and select micronutrient intakes, and Recommended Dietary Allowances (RDA) of subjects (mean ± S.D.)

Nutrient	Suboptimal Group (SG) (n=9)	Control Group (CG) (n=9)	RDA
Kilocalorie	* 1219 ± 279[a]	2126 ± 357[b]	2200.0
Protein (g)	50 ± 10[a]	71 ± 7[b]	46.0
Carbohydrate (g)	* 177 ± 42[a]	313 ± 69[b]	275.0
Fat (g)	* 34 ± 13[a]	67 ± 20[b]	73.0
Vitamin A (RE)	727 ± 198[a]	1014 ± 548[b]	800.0
Vitamin D (μg)	* 2.0 ± 1.0[a]	* 3.5 ± 1.8[b]	10.0
Vitamin E (mg)	7.7 ± 4.3	9.1 ± 3.1	8.0
Vitamin C (mg)	79.2 ± 25.2[a]	148.8 ± 70.7[b]	60.0
Thiamin (mg)	1.0 ± 0.2	2.3 ± 2.3	1.1
Riboflavin (mg)	1.2 ± 0.3[a]	1.9 ± 0.5[b]	1.3
Niacin (mg)	16.3 ± 2.9	19.6 ± 6.3	15.0
Vitamin B6 (mg)	* 1.1 ± 0.3	1.5 ± 0.6	1.6
Vitamin B12 (μg)	2.1 ± 1.5	2.7 ± 0.6	2.0
Folate (μg)	194.1 ± 61.2	257.8 ± 119.2	180.0
Calcium (mg)	* 526.8 ± 178.3[a]	* 904.6 ± 196.4[b]	1200.0
Iron (mg)	* 9.7 ± 2.1[a]	16.7 ± 9.2[b]	15.0
Zinc (mg)	* 4.9 ± 1.9[a]	* 7.3 ± 1.8[b]	12.0
Copper (mg)	* 0.74 ± 0.12	1.81 ± 1.56	2.25

[a,b] $p<0.05$ * does not meet the RDA

Table 4. Plasma and RBC zinc concentrations, and percent lysis at 0.30, 0.35, 0.40, 0.45, and 0.50% NaCl of subjects (mean ± S.D.)

Variable	Suboptimal Group (SG) (n=9)	Control Group (CG) (n=9)	Normal Values
Plasma zinc (µg/g pro)	12.24 ± 0.75	12.61 ± 0.52	
(µg/dl)	82.0 ± 8.7	78.4 ± 6.2	70 - 110 µg/dl
RBC zinc (µg/ml)	10.45 ± 1.47	10.56 ± 1.11	10.1 - 13.4 µg/dl
% lysis at 0.30% NaCl	93.8 ± 1.3	92.0 ± 3.1	97 - 100 % lysis
% lysis at 0.35% NaCl	92.8 ± 2.4	89.4 ± 3.9	90 - 99 % lysis
% lysis at 0.40% NaCl	79.5 ± 9.4	74.8 ± 10.0	50 - 90 % lysis
% lysis at 0.45% NaCl	18.6 ± 9.9	15.8 ± 6.7	5 - 45 % lysis
% lysis at 0.50% NaCl	0.43 ± 0.54	1.1 ± 1.2	0 - 5 % lysis

Table 5. IgG concentration, IgM concentration, C3 concentration, % T cells, and % Lymphocytes of subjects (mean ± S.D.)

Variable	Suboptimal Group (SG) (n=9)	Control Group (CG) (n=9)	Normal Values
IgG (mg/g protein)	164.7 ± 21.7	167.6 ± 24.9	
(mg/dl)	1102 ± 177	1047 ± 194	694 - 1618 mg/dl
IgM (mg/g protein)	24.6 ± 10.9	29.4 ± 9.1	
(mg/dl)	161 ± 66	183 ± 60	60 - 263 mg/dl
C3 (mg/g protein)	15.8 ± 2.8	15.9 ± 1.9	
(mg/dl)	105 ± 19	98 ± 10	88 - 201 mg/dl
% T cell	66.7 ± 7.8	68.3 ± 5.3	61 - 85 %
% Lymphocytes	36.7 ± 10.3	36.4 ± 9.1	15 - 41%

Table 6. Correlations of energy, macronutrient, and zinc intakes with IgG concentration.

Group	Variable 1	Variable 2	Correlation Coefficient	p value
Both (n=18)	Kcal intake	IgG	0.243	0.33
Both	Protein intake	IgG	0.289	0.25
Both	CHO intake	IgG	0.220	0.38
Both	Fat intake	IgG	0.137	0.59
Both	Zinc intake	IgG	0.267	0.28
SG (n=9)	Kcal intake	IgG	0.221	0.57
SG	Protein intake	IgG	0.616	0.07
SG	CHO intake	IgG	0.136	0.73
SG	Fat intake	IgG	0.023	0.95
SG	Zinc intake	IgG	0.328	0.39
CG (n=9)	Kcal intake	IgG	0.423	0.25
CG	Protein intake	IgG	0.127	0.74
CG	CHO intake	IgG	0.351	0.35
CG	Fat intake	IgG	0.193	0.62
CG	Zinc intake	IgG	0.240	0.53

Table 7. Correlations of energy, macronutrient, and zinc intakes with IgM concentration.

Group	Variable 1	Variable 2	Correlation Coefficient	p value
Both (n=18)	Kcal intake	IgM	0.590	0.01
Both	Protein intake	IgM	0.355	0.15
Both	CHO intake	IgM	0.555	0.02
Both	Fat intake	IgM	0.502	0.03
Both	Zinc intake	IgM	0.481	0.04
SG (n=9)	Kcal intake	IgM	0.864	0.0008
SG	Protein intake	IgM	0.266	0.56
SG	CHO intake	IgM	0.824	0.005
SG	Fat intake	IgM	0.730	0.02
SG	Zinc intake	IgM	0.487	0.18
CG (n=9)	Kcal intake	IgM	0.605	0.08
CG	Protein intake	IgM	0.339	0.37
CG	CHO intake	IgM	0.496	0.17
CG	Fat intake	IgM	0.322	0.39
CG	Zinc intake	IgM	0.354	0.35

Table 8. Correlations of energy, macronutrient, and zinc intakes with C3 concentration.

Group		Variable 1	Variable 2	Correlation Coefficient	p value
Both	(n=18)	Kcal intake	C3	0.133	0.60
Both		Protein intake	C3	0.246	0.32
Both		CHO intake	C3	- 0.037	0.88
Both		Fat intake	C3	0.290	0.24
Both		Zinc intake	C3	0.290	0.24
SG	(n=9)	Kcal intake	C3	0.380	0.31
SG		Protein intake	C3	0.475	0.19
SG		CHO intake	C3	0.106	0.78
SG		Fat intake	C3	0.597	0.08
SG		Zinc intake	C3	0.510	0.16
CG	(n=9)	Kcal intake	C3	0.050	0.90
CG		Protein intake	C3	0.182	0.64
CG		CHO intake	C3	- 0.263	0.49
CG		Fat intake	C3	0.277	0.47
CG		Zinc intake	C3	0.101	0.79

Table 9. Correlations of energy, macronutrient, and zinc intakes with % Lymphocyte.

Group		Variable 1	Variable 2	Correlation Coefficient	p value
Both	(n=18)	Kcal intake	% Lymph	- 0.011	0.97
Both		Protein intake	% Lymph	0.246	0.33
Both		CHO intake	% Lymph	- 0.068	0.79
Both		Fat intake	% Lymph	0.091	0.72
Both		Zinc intake	% Lymph	0.240	0.34
SG	(n=9)	Kcal intake	% Lymph	0.354	0.35
SG		Protein intake	% Lymph	0.647	0.05
SG		CHO intake	% Lymph	0.184	0.63
SG		Fat intake	% Lymph	0.405	0.27
SG		Zinc intake	% Lymph	0.575	0.10
CG	(n=9)	Kcal intake	% Lymph	- 0.308	0.42
CG		Protein intake	% Lymph	0.062	0.87
CG		CHO intake	% Lymph	- 0.288	0.45
CG		Fat intake	% Lymph	- 0.039	0.92
CG		Zinc intake	% Lymph	- 0.011	0.98

Table 10. Correlations of energy, macronutrient, and zinc intakes with % T cell.

Group	Variable 1	Variable 2	Correlation Coefficient	p value
Both (n=18)	Kcal intake	% T cell	0.322	0.19
Both	Protein intake	% T cell	0.173	0.49
Both	CHO intake	% T cell	0.344	0.16
Both	Fat intake	% T cell	0.313	0.21
Both	Zinc intake	% T cell	0.513	0.03
SG (n=9)	Kcal intake	% T cell	0.843	0.003
SG	Protein intake	% T cell	0.178	0.65
SG	CHO intake	% T cell	0.819	0.005
SG	Fat intake	% T cell	0.805	0.007
SG	Zinc intake	% T cell	0.632	0.06
CG (n=9)	Kcal intake	% T cell	- 0.068	0.86
CG	Protein intake	% T cell	0.019	0.96
CG	CHO intake	% T cell	0.103	0.79
CG	Fat intake	% T cell	- 0.093	0.81
CG	Zinc intake	% T cell	0.433	0.24

Table 11. Correlations of zinc intake with plasma and RBC zinc concentrations, and % Lysis at 0.30%, 0.35%, 0.40%, 0.45%, 0.50% NaCl.

Group	Variable 1	Variable 2	Correlation Coefficient	p value
Both (n=18)	Zinc intake	Plasma zinc	0.126	0.62
Both	Zinc intake	RBC zinc	0.014	0.96
Both	Zinc intake	0.30% NaCl	- 0.255	0.31
Both	Zinc intake	0.35% NaCl	- 0.260	0.30
Both	Zinc intake	0.40% NaCl	- 0.189	0.45
Both	Zinc intake	0.45% NaCl	- 0.321	0.19
Both	Zinc intake	0.50% NaCl	0.188	0.46
SG (n=9)	Zinc intake	Plasma zinc	0.264	0.49
SG	Zinc intake	RBC zinc	- 0.035	0.93
SG	Zinc intake	0.30% NaCl	0.619	0.07
SG	Zinc intake	0.35% NaCl	0.383	0.31
SG	Zinc intake	0.40% NaCl	- 0.073	0.85
SG	Zinc intake	0.45% NaCl	- 0.466	0.20
SG	Zinc intake	0.50% NaCl	- 0.035	0.93
CG (n=9)	Zinc intake	RBC zinc	0.015	0.97
CG	Zinc intake	Plasma zinc	- 0.514	0.15
CG	Zinc intake	0.30% NaCl	- 0.331	0.38
CG	Zinc intake	0.35% NaCl	- 0.229	0.55
CG	Zinc intake	0.40% NaCl	- 0.043	0.91
CG	Zinc intake	0.45% NaCl	0.014	0.97
CG	Zinc intake	0.50% NaCl	- 0.009	0.98

CHAPTER V

DISCUSSION AND CONCLUSIONS

In this study, chronic dieting in college women was defined as chronically consuming a suboptimal energy intake of less than 70% of the recommend energy intake established in the RDA (NRC 1989). This percentage of the RDA, used for identifying inadequate energy intake, has been used in other studies assessing adequate nutrition (Block et al. 1993, Nowak et al. 1988, Horwath 1991). Furthermore, in this study energy intake was identified as a "chronic" energy intake based on the length of the diet records (14 days) and the criteria of maintaining current body weight for the prior 12 months, to ensure stabilization of energy balance. However, the criteria did not restrict subjects from modifying their diet to gain or lose weight during the study period. The subjects could have utilized the diet recording as an excuse to "begin a diet"; thus, the chronic caloric intake would have been underestimated. To control for this possibility, all subjects were asked to maintain their "normal" diet throughout the study period.

Mean energy intake of the CG was 100% of the recommended energy intake, meeting the definition of an optimal energy intake. Mean energy intake of the SG was 51% of the recommended energy intake, more than meeting the definition of a suboptimal energy intake. To ensure that the energy intakes in the SG were inadequate to meet total energy needs, total energy expenditure was matched between the two groups. Matching energy output excludes the possibility of the SG having a lower energy requirement than the CG.

Although nonsignificant, the SG was slightly heavier than the CG and had a significantly greater percent body fat than the CG. Since both groups were approximately the same height and had similar estimated EEA, it can be assumed that the increased body fat contributed to the higher weight in the SG. Higher weights, associated with dieting, have been observed in other studies (Hill et al. 1992, Field et al. 1993). Hill and colleagues (1992) reported higher weights in dieting 9 and 14 year old girls as compared to nondieting girls, while Field and colleagues (1993) reported higher weights in high school dieters compared to nondieters. These studies imply that dieting is not resolving the weight problem in women. Yet, weight and body dissatisfaction may perpetuate chronic dieting. Body dissatisfaction was demonstrated in this study, where more than three fourths of the SG reported feeling overweight and dissatisfied with their body shape; but only one fourth of the CG felt the same way. This is similar to the results found in other studies (Connor-Greene 1988, Moore 1988). Therefore, body esteem appears to be a significant determinant of chronic dieting in college women.

Chronic energy restriction reduces resting energy expenditure, a measure used for determining total energy output and percent of predicted REE (Molé 1990). The influence of energy restriction on REE was demonstrated in this study where the SG had a slightly lower, yet nonsignificant, percent of predicted REE as compared to the CG. However, lean body mass also influences REE; and, in this study the CG had more lean body mass than the SG which would have increased their measures of REE.

Assessment of the eating habits of the two groups yielded somewhat surprising results. A greater percentage of the SG reported eating breakfast

every day and eating more frequently after 8pm than was reported by the CG. This conflicts with the findings of Hernon and colleagues (1986). They assessed meal frequency in dieters and nondieters and determined that the dieters ate less frequently than the nondieters. Since in this study the SG consumed less total calories, it is possible that the SG consumed smaller, more frequent meals as a mechanism to control weight. However, since meal size was not assessed, this theory is unsupported.

Several studies have shown that intakes of macronutrients and micronutrients decrease proportionally with decreasing energy intake (Bueckle et al. 1993, Hernon et al. 1986). This effect was seen in this study where the intakes of all macronutrients and several micronutrients were significantly lower in the SG as compared to the CG. In addition to this observation, several nutrient intakes in the SG fell short of meeting the RDA. Those included energy, carbohydrate, fat, vitamin D, vitamin B6, calcium, iron, and zinc. Yet, in the CG, only vitamin D, calcium, and zinc were below the recommendations. Perhaps more important, many more nutrients were found to be inadequate (<70% RDA) in the SG as compared to the CG. Similar results have been found in other studies assessing the nutrient adequacy of college women's diets (Block et al. 1993, Nowak et al. 1988). This suggests that college women are dieting by reducing caloric intake, but that they fail to compensate for the loss of food quantity by improving food quality. This dieting technique is putting these women at risk for developing multiple nutrient deficiencies.

Since vitamin D can be obtained through sun exposure, it is interesting to note that the only nutrient found to be inadequate in the diets of both the

SG and the CG was zinc. As discussed previously, zinc is one of the essential trace elements that has been repeatedly shown to be deficient in the diets of college women (Hernon et al. 1986, Murphy et al. 1986). In the SG, mean zinc intake was determined to be only 41% of the RDA. In the CG, mean zinc intake was only 61% of the RDA. Therefore, it would be assumed that the zinc status of both groups would be severely compromised if the dietary zinc assessments were accurate and reflected a chronic intake. In this investigation, plasma zinc, RBC zinc and RBC fragility were the measures used to assess zinc status. As previously described, plasma and RBC zinc content have been shown to adequately reflect zinc status in a chronic zinc-depleted state. Since this study assumed a chronic intake of nutrients, it would be assumed that the inadequate zinc intake would lead to a deficient zinc status. However, zinc status, as assessed by these biochemical and functional measures, did not appear to be compromised in either group. In both groups, the reported values for plasma zinc and RBC zinc content were within normal ranges reported by Wada et al. (1983), yet the means fell at the lower end of both ranges. In addition, % lysis at the varying NaCl concentrations were also within the reported normal ranges (Baker et al. 1962). Furthermore, zinc status, as assessed by these measures, was not significantly difference between the two groups, even though the CG consumed significantly more zinc than the SG.

The conflict between these results and other studies could be due to several reasons. The primary reason would be that the biochemical and functional measures of zinc used in this study were not sensitive enough to determine zinc status. That theory has been supported in several reviews

assessing the adequacy of plasma and RBC zinc content as measures of zinc status (Aggett et al. 1995, Prasad 1985a). These indicators have been determined to be insensitive in an acute zinc deficient state. However, in this study the assumption was made that the assessed suboptimal zinc intake represented chronic intake. With this assumption, the results of this study contradict the findings of other researchers that have observed declines in plasma and RBC zinc content following chronic zinc-deficient diets (Prasad et al. 1978, Ruz et al. 1992).

Additionally, it is possible that the zinc intakes of the subjects were underestimated. Software programs for conducting nutritional assessments of micronutrient intakes are often incomplete. Zinc intake could have been adequate in this population, yet only determined to be inadequate because of the chosen assessment tool. However, as stated previously, zinc is one of the essential nutrients most often found to be deficient in the diet of college women. Therefore, even if zinc intake was underestimated in the dietary analysis, it is likely that the subjects would have still been found to consume inadequate zinc intakes.

A final explanation for the discrepancy in the results could lie in the RDA for zinc. The recommended intake for zinc in young women has declined in the last few editions of the RDA to a current recommendation of 12 mg per day. Therefore, it is possible that the current recommendation for zinc may still be much higher than "true" zinc requirements. In this study, a lower zinc recommendation would indicate that the zinc intakes (as a percentage of the RDA) were more adequate in both groups and, thus, would better coincide with the measures of zinc status. Additional support for this

theory is in the lack of overt zinc deficiency symptoms seen in this population, which is considered at high risk for zinc deficiencies.

Immune system status in the two groups was determined by measuring the concentration of several immune components in the blood. Immune system status, based on these measures, was not found to be significantly different between the two groups. Furthermore, mean levels of the individual immune groups were within the normal levels determined for each group. In addition, both groups reported equal frequencies of illness in the prior year. These findings imply that immune system status and apparent health status is unaffected by a chronic suboptimal energy intake. However, these results conflict with the findings of Nieman et al. (1996) and Kelley et al. (1994). Both studies demonstrated significant decreases in these immune parameters following an acute caloric restriction in obese or overweight women. However, the women in the current study were at a normal weight and the caloric restriction was chronic. Therefore, since the immune system is the essential body defense unit, it is possible that the immune system of the SG adapted to the chronic suboptimal energy intake without compromising immune system functions. In PEM, a chronic severely malnourished state, the immune system is compromised. Although acute caloric restriction impairs immunity, a chronic moderate caloric restriction may not impair immunity, especially if the immune system is able to adapt to a lower nutrient intake.

An interesting relationship between energy and macronutrient intakes and the concentration of the immune components in the SG was observed in this study. In the SG, significant positive correlations were found among

intakes of energy and several macronutrients and blood levels of the immune cells and proteins, yet this effect was not observed in the CG. In addition, this effect was somewhat observed when the groups were combined. However, the significance of these correlations occurring between energy and macronutrient intakes and the immune system components when the groups were combined is somewhat questionable. It is possible that the highly significant correlations observed in the SG contributed almost exclusively to the determination of correlation significance when the groups were combined. Thus, likely, only of true significance is the relationship that was observed between nutrient intakes and immune cells and proteins in the SG.

The positive correlations observed between the nutrient intakes and the immune cell and protein concentrations in the SG could possibly indicate that below a certain energy level, select biochemical indicators of immune system status become reflective of energy, macronutrient, and/or zinc intake. However, it is important to note that this effect does not appear to alter immune status or frequency of illness. Particular immune system components that appear to be the most sensitive to changes in nutrient intakes during a suboptimal energy intake are the following: IgM concentration, which appears to be sensitive to caloric intake, carbohydrate intake, and fat intake; T cells, which appear to be sensitive to caloric intake, carbohydrate intake, fat intake, and zinc intake; and lymphocytes, which appear to be sensitive to protein intake.

It is difficult to assess which nutrient or nutrients influenced the immune components in the SG. As stated previously, decreases in energy

intake are associated with proportional decreases in individual nutrient intakes. Therefore, the correlations observed among the energy and macronutrient intakes and the immune cell and protein levels could have been a result of either the individual nutrient's apparent influence on the immune components, or as a indirect effect of the macronutrients proportionally increasing and decreasing with caloric intake. Nevertheless, there does seem to be a correlation between caloric intake at suboptimal levels and select immune cell and protein concentrations. If the immune system is able to adapt to a suboptimal energy intake, it is possible that the adaptation led to the immune system becoming more sensitive to nutrient intakes; however, this assumption is unsupported.

It is also of interest to note that protein was the only macronutrient observed to have the least number of correlations with the immune components in the SG. In the immune system, like many other systems, protein is the most critical macronutrient to the system. Thus in an energy deficient system, protein may be conserved for it's roles in immune functions, as opposed to supplying energy for sustaining body functions. Therefore, it is possible that in a chronically energy-deficient system, the immune system becomes more sensitive to intakes of carbohydrate and fat, as opposed to intake of protein.

Although no correlations were assessed between micronutrient intakes (other than zinc intake) and any of the immune components in either the SG or the CG, it could be postulated that the micronutrient intakes influenced the immune levels in the SG. However, results from the studies of Nieman et al. (1996) and Kelley et al. (1994) support the assumption of the

macronutrients alone influencing the immune cell and protein concentrations in the SG. In both studies, similar caloric restricted diets were employed at levels of approximately 1200 kilocalories per day; yet, in the study by Kelley et al. (1994) micronutrients were supplemented in the diet. Depressed immune functions were seen in both studies, suggesting that the macronutrients may have had a greater influence on the immune system than the micronutrients. In both studies, caloric restriction was acute. Therefore, the possibility of a micronutrient deficiency developing in the restriction period would not be likely. Yet, in this study, energy intakes were assumed to be chronic, and thus, an established deficiency of one or more of the micronutrients could have affected the immune components in the SG. The significant correlations observed between zinc intake and concentrations of T cells and lymphocytes in the SG reflect this possibility.

A limitation in this study was the use of college women interested in nutrition and health. Assessing women who already have an interest in nutrition and/or health may not be representative of the normal population of college women. Therefore, in future studies, a more representative population may better reflect the true nutritional and health status in college women.

A second limitation in this study pertained to the use of the subject's perceptions in determining several assessments in the study. Although instructions and diagrams were supplied to increase the accuracy of the diet records, no direct indication of compliance was available. In addition, a period of 14 days for diet and activity records may have been exhaustive for subjects; thus, the accuracy of these records may have declined over the 14

days. Subjects were asked to keep records of intentional activity for the assessment of EEA. Subjects were asked to report all activities in which they "felt like they were raising heart rate". Therefore, the subject's perception of activity greatly influenced their determined EEA. The subject's perceptions also were used in the health status assessment. Prior to blood collections, subjects were asked specifically if they had experienced an illness or any common symptoms associated with an illness. Therefore, the reported health status prior to the blood collections was based solely on the subject's perception of their health.

One essential limitation should be noted regarding the assessment of % T cells. T cells were not assessed for viability in this study. The importance of this limitation is that it would be possible to obtain a value of %T cell with nonviable cells. However, T cells are not often found to be nonviable unless a severe disease or illness is present. In this study, potential subjects were excluded from participation if they had a history of a chronic disease or illness. Therefore, although most likely insignificant, it was only assumed that the T cells assayed in this investigation were viable.

Another limitation that warrants further research is the assumption that RBC fragility was reflective of zinc intake alone and, thus, would be representative of zinc status. As stated previously, although zinc is necessary for the maintenance of RBCs, it is not the only nutrient essential for the maintenance of RBCs. Also, RBC fragility may not adequately represent zinc status in a mild zinc deficiency if it is insensitive to moderate changes in RBC membrane zinc concentration. Therefore, RBC fragility has the potential for use as an indicator of zinc status; however, it would not be a specific indicator

of zinc deficiency unless all other micronutrients involved in the cell membrane were determined to be adequate; and, it would not be a sensitive indicator of zinc status in moderate zinc deficiencies.

A major limitation in assessing immune system status is the effects of other factors on immunity. In this study, nutritional status was the only factor addressed that has been shown to influence immune status. However, numerous other factors have been associated with an altered immune status. Some of those factors include illness, stress, alcohol consumption, oral contraceptives, and caffeine consumption. None of these factors were controlled for in the present study and many would be difficult to control for at all.

In addition to the research suggested in regards to resolving the limitations in this study, several major research areas desire attention. Better methods for identifying women with chronic suboptimal energy intakes need to be established. In this study chronic intake was identified through assessing 14 days of diet records in association with a zero energy balance (determined by the maintenance of body weight). Yet, as stated previously, the subjects were not refrained from changing their caloric intake during the study, therefore it is possible that the defined suboptimal or optimal energy intakes were only acute and not chronic as assumed. Possibly utilizing other indicators of a true chronic caloric restriction such as assessing metabolic or endocrine hormones, or assessing diet records periodically would better define a group as inadequate or adequate in energy intake.

The characteristics of the SG reflected some of the characteristics which are seen in restraint eaters. Similar characteristics of restraint eaters and the

SG included a chronic caloric restriction and a heavier weight (as compared to nonrestraint eaters or the CG). However, binge eating episodes observed in restraint eaters was not observed in any of the diet records of the SG. In addition, the restrained eating questionnaire was not employed in this study. Therefore, additional research is needed to distinguish between those dieters who can be classified as restraint dieters with bingeing episodes, and those dieters (as identified in the SG) who chronically consume an inadequate energy intake without bingeing episodes.

Future studies should also address the specific effects of moderate macronutrient deficiencies on the immune system. Because individual macronutrients decrease proportionally with a decrease in energy intake, it is difficult to determined which specific macronutrients are most critical to the immune system in a suboptimal energy intake. Identifying how macronutrient intakes affect immunity in an inadequate energy intake would contribute significantly to the understanding of the roles of macronutrients in immunity. In addition, more research is needed to identify the effects of moderate micronutrient deficiencies on immune system functions. In this study, it was shown that zinc intake was associated with T cell and lymphocytes concentrations in an energy deficient state. Therefore, because of it's multiple functions in immunity, it would be important to determine the effects of moderate zinc deficiencies on the immune system, in addition to determining the effects of moderate deficiencies of other micronutrients on immunity.

CONCLUSIONS

College women, chronically dieting to lose weight, are at risk for developing moderate nutrient deficiencies because of inadequate nutrient intakes. Yet, little is known regarding the implications of moderate nutritional deficiencies. Therefore, this study was conducted to investigate the possible nutritional and health implications resulting from inadequate nutrient intakes in college women dieters. Several conclusions can be made from the results of this investigation. Foremost, nutrient intakes in women, that are actively restricting calories to lose weight, are not sufficient to meet their nutritional requirements. Thus, there is the potential in these women to develop nutritional deficiencies, which may then have severe ramifications in impairing functions. In addition, it appears from this research that college women have the potential for developing zinc deficiencies, because of inadequate zinc intakes, regardless of energy intake. Yet, this study also demonstrated the current lack of sensitive and specific indicators for determining zinc status. Even though insufficient nutrients in the diet of college women implies the potential for developing nutritional deficiencies, from this study it does not appear that the overall immune defense system and subsequent health status are affected. Yet, it does appear that the immune system becomes more sensitive to nutrient intakes when energy intake is inadequate. Future research should focus on assessing this relationship between moderate nutrient deficiencies and immunity and determining the potential implications for the current and future health status of these women.

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APPENDIX A

FLYER

Nutrition, Exercise, and Women's Health Study

LOOKING FOR WOMEN AGE 19-24 TO PARTICIPATE IN A NUTRITION STUDY

MUST HAVE MAINTAINED CURRENT BODY WEIGHT (WITHIN 5 LBS) FOR AT LEAST ONE YEAR

FOR YOU:

NUTRITIONAL ANALYSIS
*
BODY COMPOSITION MEASURE
*
BASAL METABOLIC RATE
*
AND MORE...

For More Information Contact:

Tracey @231-7708/552-8699
(email trwagner@vt.edu)

or

LeaAnn @953-3480
(email lethomps@vt.edu)

APPENDIX B

INITIAL INTERVIEW

General Information

Date _____
Name _____ Code Number _____
Current Address _____
Permanent Address _____
Current Telephone _____
Permanent Telephone _____
Social Security Number _____
Date of Birth _____
Education Level
_____ Fresh __ Soph _____ Jr _____ Sr _____ Grad

Weight History

Current Weight _____
Current Height _____
Highest Weight Since Age 12 _____
Lowest Weight Since Age 12 _____
How long did you remain at your lowest weight? _____
Desired Weight _____

At your current weight do you feel that you are:

Extremely Thin	Somewhat Thin	Normal Weight	Somewhat Overweight	Extremely Overweight
1	2	3	4	5

How dissatisfied are you with the way your body is proportioned?

Extremely Dissatisfied	Very Dissatisfied	Moderately Dissatisfied	Slightly Dissatisfied	Not at All Dissatisfied
1	2	3	4	5

Code Number_____

How often do you weigh or measure your body size?

_____more than daily _____daily
_____more than weekly _____weekly
_____less than monthly _____monthly

Dieting History

Have you ever been on a diet? _____Yes _____No
If yes, approximately how many diets have you been on since you began dieting?

____0-5 diets ____11-20 diets
____6-10 diets ____more than 20 diets

Have you ever been diagnosed with an eating disorder?
_____Yes _____No
If yes, at what age were you diagnosed?_____

Circle all that apply:

I consume large amounts of food.

Never Rarely Sometimes Often Always

I eat very rapidly.

Never Rarely Sometimes Often Always

I feel out of control when I eat.

Never Rarely Sometimes Often Always

I get uncontrollable urges to eat and eat until I feel physically ill.

Never Rarely Sometimes Often Always

Code Number_____

During the entire last month, what is the average frequency with which you have engaged in the following behaviors? (check one for each behavior)

	Never	Once a month	More than once a month	Once a week	Several times a week	Once a Day	More than once a day
Binge eating							
Vomiting							
Laxative use							
Use of diet pills							
Use of Enemas							
Exercise for weight loss							
Fasting for an entire day							
Greatly decreased intake							

Menstrual History

At what age did you start you period?_____

Have you had a regular (once a month) menstrual cycle in the past year?

_____Yes _____No

If no, estimate how many times in the past year you have had a menstrual cycle._

Are you currently using birth control pills?

_____Yes _____No

Code Number_____

Medical History

Do you have a history of a chronic disease?

_____Yes _____No

Have you been diagnosed and/or treated for a disease or severe illness in the past year?

_____Yes _____No

If yes, please describe._____

Have you been hospitalized in the past year?

_____Yes _____No

If yes, please describe._____

Please estimate how often you have been ill in the past year? (including flu, cold, allergies, strep throat, etc)

_____more than once a week
_____more than once a month
_____more than once a year
_____less than once a year

_____weekly
_____monthly
_____yearly

Have you taken any prescription or illicit drugs in the past year?

_____Yes _____No

If yes, please identify._____

Please estimate how often you have used over-the-counter medicine (including diet aids, supplements, pain relievers, illness medication, etc.) in the past year.

_____more than once a day
_____more than once a week
_____more than once a month
_____more than once a year

_____daily
_____weekly
_____monthly
_____once a year

Please list any over-the-counter medicine (including diet aids, supplements, pain relievers, illness medication, etc.) that you use **more than once a week**.

_____

_____

Code Number _____

Exercise History

How often do you exercise in a week?

_____0 times/wk
_____1-3 times/wk
_____4-6 times/wk
_____7-10 times/wk
_____11 or more times/wk

On average, how long are your exercise sessions?

_____less than 20 minutes/ session
_____20 minutes to 1 hour / session
_____1 hour to 2 hours / session
_____greater than 2 hours/ session
_____I do not exercise regularly

What is you main activity for **aerobic** exercise?_____
_____

Does you normal exercise routine include **anaerobic** activity?

____Yes _____No

If yes, what is your main activity for **anaerobic** exercise?_____
_____

What percentage of your exercise routine is **anaerobic** activity. __0-25%
25-50% ____50-75% _____75-100%

Code Number_____

24 Hour Dietary Recall

Food (description)	Amount	Size
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		
13.		
14.		
15.		

24 Hour Recall Related questions

Is this the **amount** you usually eat in a 24 hour period?

_____Yes _____No

If no, do you usually consume more or less?

_____More _____Less

Is this the **type** of food you usually eat every day?

_____Yes _____No

Do you normally eat breakfast? _____Yes _____No

How many days in a week do you eat after 8 pm?

_____0 days/wk

_____1-2 days/wk

_____3-4 days/wk

_____5 or more days/wk

Code Number_____

What is you largest meal of the day?

_____Breakfast

_____Lunch

_____Dinner

_____Other, please specify_____

APPENDIX C

INFORMATION ABOUT THE EATING DISORDERS

Because of the nature of this investigation and the high prevalence of clinical eating disorders among women of college age, it is our obligation to inform you of the support services available to Virginia Tech students in the event that you or someone you know should need assistance.

The eating disorders, Anorexia Nervosa, and Binge Eating Disorder, are not about weight loss or weight maintenance. Each disorder involves an intricate relationship with food which goes beyond eating for sustenance. The young woman with an eating disorder may begin her journey into self-destructive behavior with the intent to simply "lose a few pounds". Underlying psychological and emotional stresses, coupled with what is sometimes referred to as a "spiritual emptiness", turns simple weight loss into an obsessive desire for perfection and control over what may be a very chaotic life. The woman with Anorexia Nervosa is able to use this control to restrict her food intake and thus maintain an outward appearance of perfection. The Bulimic, unable to control her food intake, finds herself bingeing on large amounts of food and then ridding herself of the food through self-induced vomiting or laxative abuse. The woman with binge eating disorder, like the bulimic, consumes large amounts of food when she is bored, tired, angry, or lonely, however, she does not purge.

It is quite common for young women to engage in behaviors associated with the eating disorders in the quest for thinner bodies. Many women on occasion limit their food intake or take diet pills when they feel they must shed some weight. The difference between these women and those with clinical eating disorders lies in the frequency and the severity of these behaviors and the obsessive fear of becoming overweight.

Should you or someone you know need assistance, please contact one of the following student focused support services:

Center for Family Services	231-7201
Psychological Services Center	231-6914
University Counseling Services	231-6557
Campus Ministries	231-3787

APPENDIX D

INFORMED CONSENT FORM

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Informed Consent for Participants of Investigative Projects

Title of Project: **Physiological and Metabolic Effects of Chronic Suboptimal Nutrition.**

Investigators: **Elizabeth A. Thomas, Ph.D.**
Shala E. Davis, Ph.D.
LeaAnn E. Fritsch
Tracey L. Wagner

I. The Purpose of this Research

It is well known that a severe deficiency of nutrients will impair human function. Most people in the U.S. do not have severe nutrient deficiencies. However, many groups in the U.S. are consistently found to consume less than the recommended levels of many nutrients. It is unclear at what point a reduction in nutrient intake will lead to detrimental effects in humans. However, based on known functions of nutrients, it can be speculated that chronic marginal nutrient deficiencies would lead to significant health concerns. This study is being conducted to identify possible consequences of chronic consumption of energy levels below recommended levels.

II. Procedures

Dietary intake and activity levels will be assessed from daily records completed by participants. Resting Metabolic Rate (RMR) will be measured by indirect calorimetry. This will require participants to come to the Laboratory for Health and Exercise Science (LHES,WMH 230) in the morning, after a 12 hour fast. After waking in the morning, they will come to the LHES and rest lying down for 30 minutes. Immediately following rest, they will have their oxygen consumption measured for approximately 10 minutes. This will require participants to wear a noseclip and mouthpiece.

Two, 50 ml (<4Tbs.) venous blood samples will be collected approximately 3 to 4 days apart, each following a 10 hour fast. Blood collections will be performed in Wallace Hall on the Virginia Tech campus.

An exercise protocol will include a total of three visits to the LHES. The first session will serve as an orientation to the laboratory and equipment, and will include an assessment of percent body fat using skin fold calipers. The second session will be used to assess maximal capacity to exercise on a cycle ergometer. This assessment will begin with cycling at no load which will be increased by 30 watts every 2 minutes until fatigue is reached. Maximal level will be defined by oxygen consumption levels. The third session will require subjects to exercise on a cycle ergometer for 30 minutes at a moderate intensity. During this supervised exercise session, the subjects will be monitored for heart rate, gas exchange and ratings of perceived exertion.

Approximate time required for participation in the research protocol is anticipated to be as follows:

Completion of diet and activity records- 20 minutes per day for 14 days
Completion of health symptoms and menstrual cycle charts- 5 minutes per day for 30 days
Measurement of RMR- 1 hour (one time only)
Blood collection- 1 hour (two times, 3-4 days apart)
Exercise protocol- session one (including body fat analysis)- 30 minutes
session two- 2 hours
session three- 2 hours

III. Risks

Venous blood collection occasionally results in bruising. Some subjects may experience distress during blood draws. Every effort will be made to assure the comfort of the subject. No coercion will be used to persuade subjects that are reluctant to participate. Juice and snack will be provided for the subjects following the blood collections. There are no risks associated with RMR or body fat measurements. Possible discomforts associated with the exercise protocol include leg fatigue, muscle soreness, a dry mouth (from a mouthpiece used to collect expired gases), and the regular risks associated with physical activity.

IV. Benefits of this Project

Benefits of participation include the provision of nutritional status information and assessment based on the biochemical and dietary analysis. Also, participants will receive information on their level of functional capacity and basal metabolism. No promise or guarantee of benefits have been made to encourage you to participate.

V. Extent of Anonymity and Confidentiality

The results of this study will be kept strictly confidential. All information and samples will be coded with subject numbers. In any publication or presentation of the results of this study, subjects will be referred to by code. The principal investigators and graduate research assistants will be the only individuals that will have access to the data and codes.

VI. Compensation

No compensation for subject participation is provided.

VII. Freedom to Withdraw

Subjects are free to withdraw from the study any time without penalty. Subjects are free not to answer any questions or respond to experimental situations that they choose without penalty.

VIII.Approval of Research

This research project has been approved, as requested, by the Institutional Review Board for Research Involving Human Subjects at Virginia Polytechnic Institute and State University.

IX. Subject's Responsibilities

I voluntarily agree to participate in this study. I have the following responsibilities:
Accurately complete dietary intake and activity records for 14 days
Accurately complete health symptoms and menstrual cycle charts for 30 days
Eat and drink nothing except water for 12 hours prior to RMR measurement
Participate in a measurement of my oxygen consumption as an indicator of RMR
Attend and participate in three sessions of the exercise protocol and body fat analysis
Eat and drink nothing except water for 10 hours prior to blood collections
Provide two, 50 ml samples of venous blood
Sit quietly for approximately 10 minutes after blood draws

X. Subjects Permission

I have read and understand the Informed Consent and conditions of this project. I have had all my questions answered. I hereby acknowledge the above and give my voluntary consent for participation in this project.

If I participate, I may withdraw at any time without penalty. I agree to abide by the rules of this project.

_____ _____

Signature Date

Should I have any questions about this research or its conduct, I may contact:

Elizabeth Thomas, Ph.D., R.D. Assistant Professor	540-231-8763
Shala E. Davis, Ph.D. Assistant Professor	540-231-8320
E.R. Stout Chair, IRB Research Division	540-231-9359

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MEMORANDUM

TO: Elizabeth Thomas, Shala Davis, LeaAnn Fritsch, Tracey Wagner
Human Nutrition and Foods

FROM: H. T. Hurd
Director

DATE: September 16, 1996

SUBJECT: IRB EXPEDITED RE-APPROVAL/"Physiological and Metabolic Effects of Chronic Suboptimal Nutrition in Young Women" - IRB #95-254

I have reviewed your request to the IRB for the above referenced project. I concur that the activity is of minimal risk to the human subjects who will participate and that appropriate safeguards have been taken. Therefore, on behalf of the Institutional Review Board for Research Involving Human Subjects, I have given your request expedited approval.

This approval is valid for 12 months. If the involvement with human subjects is not complete within 12 months, the project must be resubmitted for re-approval. We will prompt you about 10 months from now. If there are significant changes in the protocol involving human subjects, those changes must be approved before proceeding.

Best wishes.

HTH/pli

APPENDIX E

Instructions for Diet and Activity Records

Diet Record

1. Keep a diet record for all food and beverages(except water),that you consume, every day for 14 days total (2 weeks).

2. Record each item and the amount that you had (i.e. oz, cups, tsp., etc). If appropriate, record the size of the item (i.e. sm, med, lg)

3. Hints:
   - if it is a prepared food, include the brand name. Also, amounts are usually listed on the containers (i.e. 12 oz can of soda)
   - please remember to include all "extras" such as condiments, snacks, etc (i.e. salad dressing, catsup, butter, candy, jelly)

4. **Please maintain as typical diet as possible during the 14 days** (i.e. your "normal diet")

5. Please refrain from starting any vitamin or mineral supplements during the time you are in the study.

Activity Record (intentional activity only)

1. keep a record of all intentional activity done for the same 14 days that you are doing the diet records.

2. Record all activities individually (i.e. wt training, aerobics, cleaning)

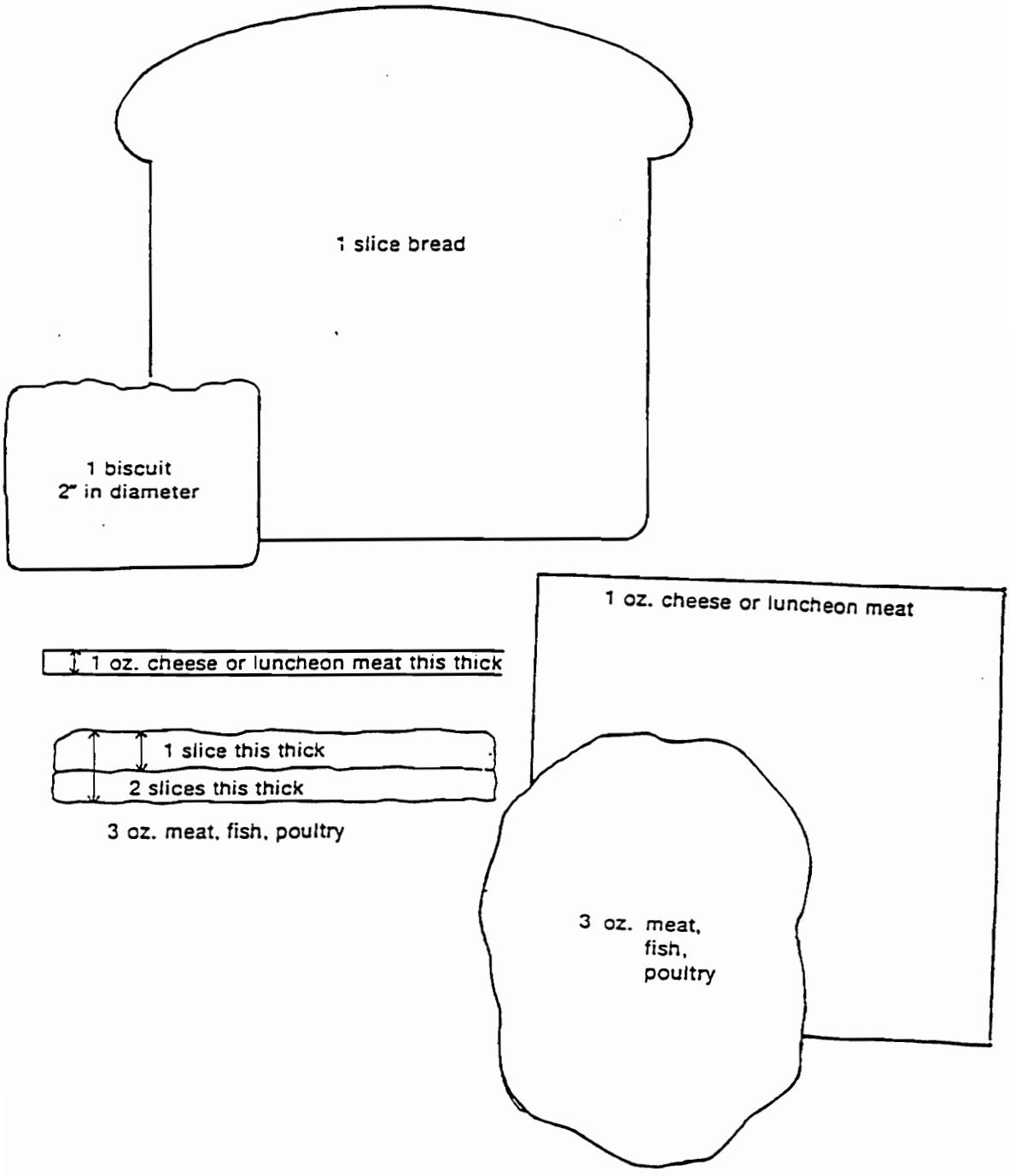
3. If you are doing an extended activity (such as skiing, waitressing, hiking, etc) estimate and record only the amount of time that you were actually physically active and do **not** include any time that was spent where you were not physically active (ie, standing, waiting, resting, etc)

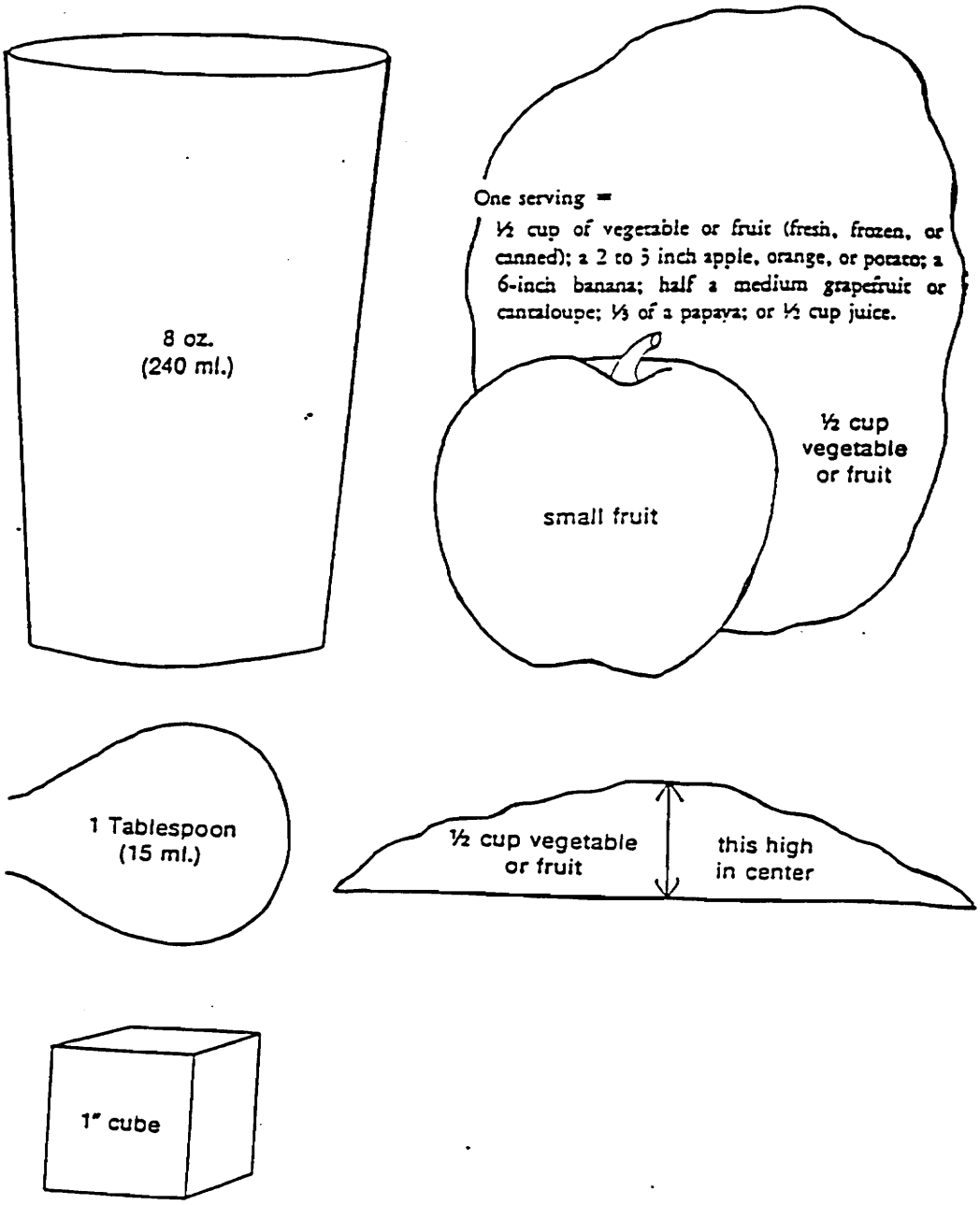
4. If you walk between classes:
only include walking between classes as an activity, if you are walking more that 15 minutes at a time for at least two times per day. Please combine all the walking sessions into one activity for the particular day or recording purposes (it saves us time and paper).

If you have any questions, please feel free to call me (Tracey) and ask.
231-7708 (office)
552-8699 (home)

APPENDIX F

SERVING SIZE ESTIMATES




8 oz.
(240 ml.)
One serving =
½ cup of vegetable or fruit (fresh, frozen, or canned); a 2 to 3 inch apple, orange, or potato; a 6-inch banana; half a medium grapefruit or cantaloupe; ⅓ of a papaya; or ½ cup juice.
small fruit
½ cup
vegetable
or fruit
1 Tablespoon
(15 ml.)
½ cup vegetable
or fruit
this high
in center
1" cube

APPENDIX G

DAILY RECORD OF FOOD INTAKE- EXAMPLE

As accurately as possible, record all food and beverages (except water) you consume each day and the amount (quantity and size) consumed. When necessary, include a description or preparation of the food or beverage. An example is provided below.

Date _____ Code Number _____

Food (description and/or preparation)	Quantity	Size
cinnamon raisin bagel	1 whole	large
orange juice (freshly squeezed)	8 oz	n/a
pizza (thin crust,sauce,cheese, mushrooms, onions)	3 slices/ Lg pizza	n/a
pasta (cooked)	2oz	n/a
tomato sauce	1/2 cup	n/a
orange	1	medium
diet coke	20 oz	n/a
pretzels (hard, thin sticks)	30 each	n/a
cake (chocolate cake, chocolate icing)	1/9 of whole cake	n/a
salad- lettuce	1 cup	n/a
tomato	1/2 cup	n/a
mushrooms (fresh)	3	small
cheddar cheese	1 oz	n/a
Italian dressing (fat free)	4 Tbs	n/a
popcorn	4 cups	n/a
cereal	1 oz	n/a
milk (1 percent)	1/2 cup	n/a

Comments:

APPENDIX H

DAILY ACTIVITY LOG code _____ date_____

ACTIVITY:_____

_____

DURATION (time): _____ minutes
HEART RATE: _____ beats/minute (optional)
BREATHING RATE: circle one
light (no difficulty talking) **moderate** (slight difficulty talking)
heavy (difficulty talking) **very heavy** (very difficult to talk)
***RATE OF PERCEIVED EXERTION:** _____

date_____

ACTIVITY:_____

_____

DURATION (time): _____ minutes
HEART RATE: _____ beats/minute (optional)
BREATHING RATE: circle one
light (no difficulty talking) **moderate** (slight difficulty talking)
heavy (difficulty talking) **very heavy** (very difficult to talk)
***RATE OF PERCEIVED EXERTION:** _____

*Scale for Ratings of Perceived exertion (RPE): (how you feel)

0.5 = very, very light; just noticeable

1 = very light

2 = light

3 = moderate

4 = somewhat hard

5-6 = heavy, strong

7-9 = very hard

10 = very, very hard; almost maximum; exhaustion

COMMENTS:

APPENDIX I

EXAMPLE OF MENSTRUAL CHART RECORD

Circle the day that you begin or began menstrating (including as many past cycles as possible). Code Number_____

June

Sun	Mon	Tues	Wed	Thur	Fr	Sat
						1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29
30						

July

Sun	Mon	Tues	Wed	Thur	Fri	Sat
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30	31			

August

Sun	Mon	Tues	Wed	Thur	Fr	Sat
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	31

September

Sun	Mon	Tues	Wed	Thur	Fr	Sat
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30					

VITA

Tracey Lynn Wagner was born on August 22, 1972 in Waynesboro, Pennsylvania. In May of 1994, she graduated from Mansfield University in Mansfield, Pennsylvania with a Bachelor's degree in Biology and a Chemistry minor. In August of that year, Tracey began work on her Master of Science degree in the Department of Human Nutrition, Foods, and Exercise at Virginia Polytechnic Institute and State University. Her research interests focus on clinical nutrition, with an emphasis in laboratory research in nutrition and medicine. Following completion of her degree, Tracey plans to pursue a career in laboratory research and aspires to, one day, teach at a college-level institution.